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Determination of the Use of Solid Particle Samplers for 'Giardia' Cysts in Natural Waters

Wright State Univ., Dayton, OH. Dept. of Biological Sciences

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16. ABSTRACT

The primary objective of this study was to improve the current methodology for concentrating, recovering, and detecting cysts of *Giardia lamblia* in water supplies. Two sampling processes for the concentration of cysts were examined. One process was diatomaceous earth filtration while the second was that of cyst concentration onto charged particles. Cysts of *G. muris* were used to determine the retention efficiency of ion-exchange resins and each type of diatomaceous earth filter examined. Cyst desorption efficiencies were evaluated for ion-exchange resins that best retained cysts, while backwashing parameters were optimized for diatomaceous earth filters. Results of cyst retention experiments indicated that two processes, anion-exchange concentration of cysts and diatomaceous earth filtration, had the potential to be developed into field methods. Comparison of these two processes at low cyst inoculum concentrations (1×10^3 cysts/liter) indicated that a greater number of cysts could be recovered from the diatomaceous earth filters. When forty liter samples of tap water containing between 1.0×10^4 and 7.0×10^5 cysts were passed through diatomaceous earth filters, 5.2 to 31.1% of the cysts were recovered in the backwash. As a result, the diatomaceous earth filter was comparable to microporous filtration and may have application in sampling finished water supplies. However, its utility in raw sampling is limited since turbidity severely reduced the recovery efficiency of cysts.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a coordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides and nonionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report describes the results of efforts to determine the efficacy of using particulate filters as an alternative to the orlon fiber filter which is used to sample large volumes of water for Giardia lamblia cysts. A better understanding of alternate filtration procedures potentially would allow for development of a better sampling device. A better sampling device is necessary for routine water monitoring and for water quality regulations development.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

Parasitic flagellates in the genus Giardia are distributed worldwide and are now the most commonly reported human intestinal parasites in the United States and Britain. Twenty-three waterborne outbreaks of giardiasis affecting over 7000 people occurred in various states in the United States between 1965 and 1977. Because of this significant increase in the incidence of waterborne outbreaks of giardiasis, efforts have been made to develop reliable and/or sensitive methods to determine the presence or absence of Giardia lamblia cysts in water supplies. The primary objective of this study was to improve the current methodology for concentrating, recovering and detecting cysts of G. lamblia in water supplies.

Two sampling processes for the concentration of cysts were examined. One process was diatomaceous earth filtration while the second was that of cyst concentration onto charged particles. Cysts of Giardia muris were used to determine the retention efficiency of ion-exchange resins and each type of diatomaceous earth filter examined. Cyst desorption efficiencies were evaluated for ion-exchange resins that best retained cysts, while backwashing parameters were optimized for diatomaceous earth filters. Results of cyst retention experiments indicated that two processes, anion-exchange concentration of cysts and diatomaceous earth filtration, had the potential to be developed into field methods. Comparison of these two processes at low cyst inoculum concentrations (1×10^3 cysts/liter) indicated that a greater number of cysts could be recovered from the diatomaceous earth filters. When forty liter samples of tap water containing between 1.0×10^4 and 7.0×10^5 cysts were passed through diatomaceous earth filters, 5.2 to 31.1% of the cysts were recovered in the backwash. As a result, the diatomaceous earth filter was comparable to microporous filtration and may have application in sampling finished water supplies. However, its utility in raw sampling is limited since turbidity severely reduced the recovery efficiency of cysts.

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SECTION 1

INTRODUCTION

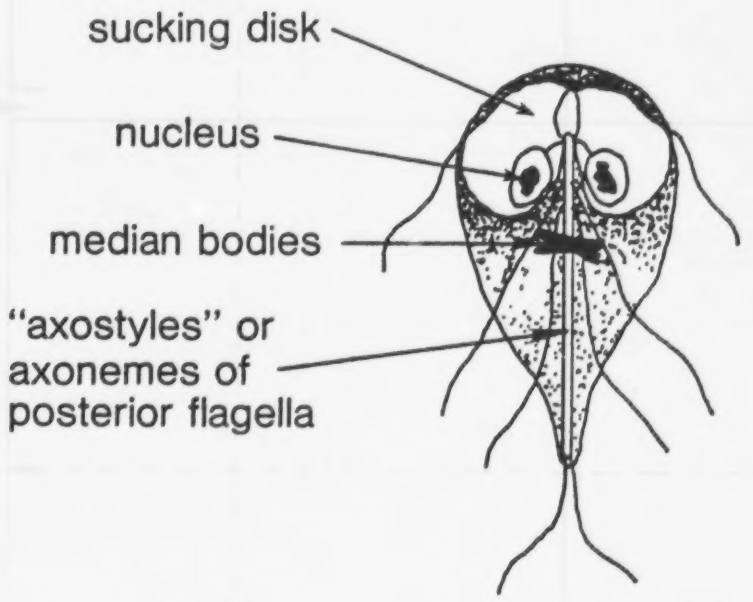
OBJECTIVES OF THE STUDY

The primary objective of this study was to improve the current methodology for concentrating, recovering, and detecting cysts of Giardia lamblia in water supplies. Two sampling processes for the concentration of G. lamblia cysts were examined. One process was diatomaceous earth filtration while the second process examined was that of cyst concentration onto charged particles. The first process was based on the hypothesis that cysts could be efficiently trapped on the surface of diatomaceous filters and subsequently recovered through backwashing the filter with a small volume of water. The second process was based on the hypothesis that cysts could be attracted to charged surfaces since they have been shown to have a charge of approximately -25 mv at pH 5.5 and to increase their electronegativity as the pH rises to 8.0 (18). In addition, charge-attraction techniques have been applied to the concentration of viruses and bacteria from water (10,11) and to the concentration of trypomastigotes (19,31) and sporozoites of Plasmodium in clinical samples (20).

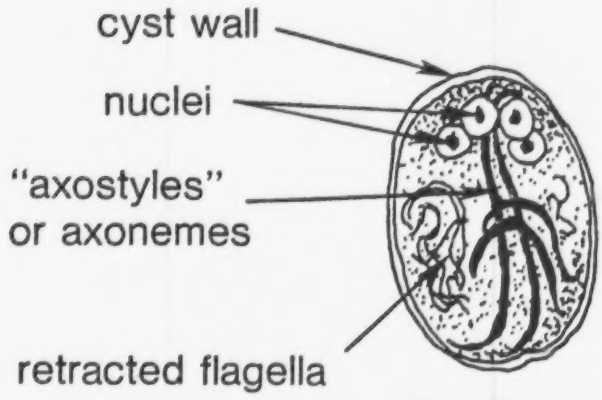
SUMMARY OF PREVIOUS WORK

Parasitic flagellates in the genus Giardia are distributed worldwide and are now the most commonly reported human intestinal parasites in the United States and Britain (28). The phases of this parasite, which are shown in Figure 1, are composed of 2 stages: the cyst stage and the trophozoite stage. Transmission of Giardia most often occurs when viable cysts are ingested directly or through water contaminated with feces. The average incubation period of giardiasis is 8 days, with a range of 3-42 days. While most infections are asymptomatic, some people have a short-lasting acute diarrheal disease, nausea, and anorexia. A small percentage develop an intermittent or protracted course characterized by diarrhea, cramping, abdominal pain, bloating, and flatulence. Diarrhea with or without overt malabsorption may last months or even years (7).

As a measure of the significance of giardiasis in the United States, twenty-three waterborne outbreaks affecting over 7000 people have been caused by this infection between 1965 and 1977 (6). Epidemic outbreaks have been reported in New York (28), New Hampshire (16), Pennsylvania (24), Colorado (15,21,22,23,30) and Washington (8). The majority of these outbreaks were attributable to public consumption of minimally treated water which was fecally contaminated. In addition, sporadic epidemics have occurred among infants and children in hospital nurseries, custodial and residential institu-



A. Trophozoite



B. Cyst

Figure 1. Morphological Stages of Giardia Lamblia

tions, and in day-care centers where personal hygiene standards were not stringent (7). Water supplies were most likely contaminated by untreated human waste or by aquatic mammal waste since asymptomatic carriers and aquatic mammals have been identified as the major reservoirs (7,9,15,17).

Because of the significant increase in the incidence of waterborne outbreaks of giardiasis reported, efforts have been made to develop reliable and/or sensitive methods to determine the presence or absence of Giardia lamblia cysts in water supplies. The major problem associated with developing sampling technology is that cysts are assumed to be present in low numbers (12), therefore necessitating the need for large-volume water sampling. During the past few years, several approaches have been taken to concentrate and detect cysts in water supplies. Developed methods can be divided into three major categories: (a) membrane filtration, (b) particulate filtration, and (c) microporous filtration.

Moore et al. (22) utilized membrane filtration to examine water and sewage samples for Giardia cysts. One and two-liter samples were passed through cheesecloth and then filtered through 0.45 um porosity membrane filters. Sediment on the filter surface was brushed into water, centrifuged and preserved in 10% formalin for microscopic examination. No cysts were observed in 10 water samples. Barbour, et al. (3) used the method of Chang and Kabler to filter 22 liters of stream water following an outbreak. However, no Giardia cysts were found. Luchtel, et al. (17) utilized 293 -mm diameter 5.0 um pore size nucleopore filters to concentrate formalin-fixed G. lamblia cysts from 20 liter tap water samples. Recovery rates of approximately 75% were found under such conditions.

The Center for Disease Control (CDC) had developed a large-volume sampling technique (14,28). The method used a swimming pool filter in which sand was the sampling medium. During an outbreak in Rome, New York, a total volume of 1.1×10^6 liters (28,000 gal) of water was collected at an average flow rate of 76 liters/min (20 gal/min) through the filter daily for 10 days. The filter backwash was collected each day in two 210 liter drums and coagulated with alum. The resulting sediment was collected and aliquots were fed to beagle puppies and examined microscopically. Two of ten samples fed to dogs produced infection and a single cyst was observed in one sample. In 1976, the U.S. EPA developed a cyst concentration technique involving the filtration of a large volume of water (100 gal or more) through a microporous orlon fiber filter. The method and development of the method has been described in detail by Jakubowski and Ericksen (12), and has been tentatively adopted as the "method of choice" for concentrating cysts from water supplies. However, the reliability and validity of the technique has yet to be fully evaluated. Limited laboratory evaluations have indicated that cyst recovery was only in the range of 3-15%, with a mean of 6.3% (12). The interpretation of positive or negative field data is uncertain. In the sampling methodology described above, efficient cyst concentration from water was possible under certain conditions. However, processing of the concentrate led to significant losses of cysts. Therefore, a major problem in detecting Giardia cysts in contaminated water supplies using any of the above techniques has been the quantitative recovery of cysts from the filter medium.

SECTION 2

CONCLUSIONS

Based on the objectives and results of this study the following conclusions can be drawn. A). Results of resin capacity and cyst retention experiments indicated that two processes, anion-exchange concentration of cysts and diatomaceous earth filtration, had the potential to be developed into field methods. B). Analysis of the data with respect to inoculum concentration indicated that a greater number of cysts were recovered from the diatomaceous earth filters when dilute samples (1×10^3 cysts/liter) were filtered. Based on these data diatomaceous earth filters were considered the best choice. C). Comparison of the efficiency of the diatomaceous earth filter to the reported efficiency of the EPA method of microporous filtration (13.0 and 6.7%, respectively) indicated that the two were similar. However, it appeared that the diatomaceous earth filter was more severely affected by the composition of the water sample than the microporous filter would be. D). The diatomaceous earth filter may have application to finished water supply sampling; however, its use on raw water samples is limited at the present time.

SECTION 3

RECOMMENDATIONS

1. The results of this study indicated that Giardia cysts could be efficiently concentrated on either the surface of diatomaceous filters or on the surfaces of charged particles. However, subsequent research should be directed toward the efficient recovery of concentrated cysts. Further research should be carried out with weak ion-exchange resins that operate effectively in a narrower pH range or with charge-modified filters similar to those used to concentrate viruses.

2. One of the major problems encountered in this study was the lack of a sensitive detection technique. Quantification of Giardia cysts is presently based upon microscopic identification and counting. Experimental studies should be carried out to develop accurate detection of small numbers of cysts mixed with other microorganisms and debris from aquatic habitats. Immuno-fluorescent techniques similar to those used for the detection of bacteria should be investigated.

3. The behavior of Giardia cysts on certain resins observed in this study raised some questions concerning the biochemical and physiological nature of cysts. Little information is available on these subjects. Basic research on the nature of the cysts themselves would lead to rational decisions on applicable sampling methods.

SECTION 4

MATERIALS AND METHODS

TEST ORGANISM

Cysts of Giardia muris were utilized throughout the study as surrogates to G. lamblia as suggested by Roberts-Thomson, et al. (26). The strain of G. muris used for this work was initially obtained from the Health Effects Research Laboratory (HERL), U.S. EPA and subsequently maintained in female ICR strain outbred mice following the methods of Roberts-Thomson, et al. (26). On one occasion the cysts were lost due to a contamination of Hexamita and a fresh inoculum was obtained from HERL.

CYST PROPAGATION

Cysts of G. muris were routinely harvested from fecal material of infected mice. Ten female ICR Strain outbred mice were orally intubated with a minimum of 10,000 cysts of G. muris. Following a five-day incubation period, mice were placed in metabolism cages and fecal samples were collected in 300-400 ml of tap water (26).

CYST ENUMERATION

Following resuspension of concentrated cyst samples, a 10 ul aliquot was taken with a micropipette (Eppendorf, Brinkman Inst. Co., Westbury, N.Y.) and placed in each of two wells of an A.O. Spencer Brightline Hemacytometer (American Optical Corp., Buffalo, N.Y.). Counts were made in the four one-millimeter square corners and center ruled area within each well of a hemacytometer at 400 x magnification with an Olympus Model CH microscope. Counts were then averaged and expressed as 10^4 cysts/ml or greater in the following manner:

$$\text{cysts/ml} = \frac{\text{No. cysts counted}}{\text{No. mm}^2 \text{ sections counted}} \times \frac{10}{1\text{mm}} \times \frac{1000 \text{ mm}^3}{1 \text{ ml}} \quad (1)$$

Whenever sample concentrations were less than 1×10^4 cysts/ml, the entire gridded area of the hemacytometer had to be counted (2).

In order to compare counts obtained from various samples, the variability associated with the use of a hemacytometer was determined. Sanford et al. (27) have shown that the distribution of particles within a hemacytometer well follows a Poisson distribution. Under such an assumption, the error

associated with any count was determined with the following equation:

$$\% E = \frac{k(CV)}{\sqrt{n}} \qquad (2)$$

Where % E = decimal error, k = a specified confidence level, n = number of wells counted and CV = coefficient of variation, numerically equal to the sample standard derivation(s) divided by the sample mean ($\bar{\chi}$). Equation (2) was rearranged in order to determine the number of replicate wells that had to be counted to insure an error of 10% or less at a 95% confidence level. Rearrangement of equation (2) yielded:

$$n = \left[\frac{k\ (CV)}{\%\ E}\right]^2 \qquad (3)$$

CYST CONCENTRATION

Depending upon the sample type, G. muris cysts were concentrated in one of two ways to a final volume of 1 or 10 ml.

Cyst Concentration from Fecal Suspensions. Fecal samples collected in 300-400 ml of tap water were mixed for 25-30 min with a magnetic stirrer and then strained through four layers of cheesecloth. The resulting liquid was centrifuged at 450 x g for 5-10 min in 50 ml conical centrifuge tubes in an IEC, International Model K Centrifuge (International Equipment Co., Needman Hts., N.Y.). The supernatant was aspirated and the resulting sediment was resuspended in approximately 10 ml distilled water, layered onto 5-6 ml of 1.1 M sucrose, then centrifuged at 450 x g for 5-15 min in an IEC Model CL centrifuge (Damon/IEC Division, Needmon Heights, Mass.). Cysts were collected at the sucrose-water interface with a Pasteur pipette, diluted to 10 ml in distilled water and washed via centrifugation two times before counting and use (26).

Cyst Concentration from Water Samples. Cysts suspended in experimental water samples (feed solutions, column effluents, backwash water, or resin washes) were concentrated on a 47 mm diameter cellulose triacetate membrane filter with a 5.0 um pore size (Gelman Sciences, Inc., Ann Arbor, Mich.) by passing the sample through the filter under negative pressure. Following filtration, the filters were carefully removed from the holder and placed into a 50 ml beaker containing 10 ml of 0.02% Tween 20 (Polyoxyethylene Soritan monolaurate, Fisher Sci.) in deionized water. Cysts were freed from the filter surface by vibrating the filter for 2 min with a "Maxi-Mix" mixer (Sybron/Thermodyne, Dubuque, Iowa) and then decanting the liquid into 15 ml conical centrifuge tubes. Following centrifugation at 450 χ g for 10 min, the supernatant was aspirated to 1 ml and the cyst pellet was resuspended.

The reproducibility of this method was measured by inoculating 20 one-

liter distilled water samples with cysts to yield final concentrations ranging from 1.0×10^5 to 1.0×10^6 cysts/ml, concentrating the suspended cysts via membrane filtration and then counting the number of cysts recovered from the filter surface. The recovery efficiency of the method was estimated by calculating 95% confidence intervals around the mean percent recovered using equation (4):

$$CI_{.95} = \bar{X} \pm \left[\frac{1.96\ [CV][\bar{X}]}{\sqrt{n}} \right] \qquad (4)$$

Where $CI_{.95}$ = the 95% confidence interval, n = number of replicate samples, $\bar{X}$ = mean % recovery of the samples, and CV = coefficient of variation.

SAMPLING MEDIUM SELECTION AND PREPARATION

Based on the objectives of the study, i.e, to develop a cyst sampling technique that had a high probability of concentrating cysts from large volumes of water on a filter and a reasonable probability of recovering concentrated cysts from the filter, a series of ion-exchange resins and diatomaceous earth were selected.

Ion-exchange Resin Selection. Recent literature has indicated that certain protozoan forms can be successfully concentrated from fluids onto ion exchange resins (19,20,31). In order to test the hypothesis that such a technique could be applied to the concentration of Giardia cysts, several ion exchange resins were initially evaluated. Since cysts of G. muris have been shown to have a net negative charge (18), two mechanisms of cyst concentration onto ion exchange resins were possible. Lumsden, et al., (19), Mack, et al. (20) and Villalta and Leon (31) have demonstrated that parasitic forms of Trypanosoma and/or Plasmodium can be concentrated from blood by anion exchange processes. However, Brewer and Bell (4) have also shown that electrostatic attractive forces may have been significant in the concentration of Amoeba proteus onto ion exchange resins. Therefore a series of anion-exchange resins and cation-exchange resins were initially screened for their ability to concentrate G. muris cysts from liquids. Two types of anion-exchange resins were selected: DEAE-Sephadex, A-25 (Pharmacia Fine Chemicals, Piscataway, N.J.) as suggested by Lumsden, et al. (19) and polystyrene-divinylbenzene anion-exchange resins (Dowex 1-X4 and Dowex 1-X8) (Bio-Rad Laboratories, Rockville Center, N.Y.). The cationic resins evaluated were also polystyrene-divinylbenzene resins (Dowex 50W-X4 and Dowex 50W-X8). All polystyrene-divinylbenzene resins were strong acid or base resins and were varied in percent crosslinkage (X4 = 4%, X8 = 8%).

Ion-exchange Resin Preparation. All ion-exchange experiments were done in columnar configuration. A known weight (1-10 gm) of each resin was washed into a column and then washed with 8-10 bedvolumes of deionized water to remove any residual ions. Resins were then washed with 3 bedvolumes of 1.5N HCl to charge the anionic resins with chloride (Cl^-) and the cationic resins with hydrogen (H^+). Each column was then backwashed with at least 3 bedvolumes of deionized water to neutrality before use (25).

Diatomaceous Earth Selection and Preparation. Three types of diatomaceous earth were initially screened for cyst retention: Celite 505, Hyflo Super-Cel and Celite 560 (Johns-Mansville, Denver, Colo.). The principle difference between each celite filter was particle size. Celite 505 was composed of particles ranging from 6 - 40 um, while Hyflo Super-Cel contained diatomaceous earth particles ranging from 2.0 - 30 um in diameter. Celite 560 was almost exlusively composed of particles greater than 40 um diameter.

Diatomaceous earth filters were prepared by placing 40 gm (dry weight) of diatomaceous earth into a plexiglass column. Diatomaceous earth particles were retained by a 15 um screen. The filter was washed with distilled water until all particles less than 15 um were removed and then backwashed to remove any remaining small particles. The column was maintained in 500 ml distilled water until use.

SCANNING ELECTRON MICROSCOPY

During the course of the study, scanning electron microscopy (SEM) was utilized to visualize the adsorption characteristics of Giardia cysts on particular resins. To avoid dislodging cysts adsorbed to bead surfaces, cyst-bead suspensions were prepared by the method of Brody and Wharton (5). Ion-exchange beads were charged with cysts of G. muris by mixing 1.0 gram (wet wt.) of each resin with 10 ml of distilled water containing 1.0×10^4 cysts/ml. The cysts were mixed with the resins by gentle inversion for 10 min and the beads were allowed to settle. The overlying water was aspirated off and replaced with a 10 ml solution of 96.6% glycerol and 3.35% KCl (wt/wt). The beads were allowed to soak for 5-10 min and then several beads were carefully removed from the suspension with forceps, cemented onto aluminum posts, and observed with a Phillips 500 Scanning Electron Microscope (12KV source, 640 Angstroms spot size: Phillips Electronics Instruments, Inc., Mt. Vernon, N.Y.).

DATA ANALYSIS

All data analysis was carried out using the Statistical Analysis System (SAS) program package (29).

SECTION 5

EXPERIMENTAL PROCEDURES

STUDY PROTOCOL

Since the study consisted of a series of experiments to evaluate the applicability of ion-exchange chromatography and diatomaceous earth filtration for Giardia cyst sampling techniques, a study protocol was designed to systematically evaluate and compare each particulate in relation to a set of criteria that would lead to the selection of a particulate best suited for the detection of Giardia cysts in water. The criteria used to evaluate each particulate included:

(a) Cysts concentration efficiency; a particulate should concentrate a large percentage of cysts in seeded samples or natural samples.

(b) Sensitivity; a particulate should be able to capture cysts in low numbers from large volumes of water.

(c) Cyst recovery; cysts concentrated onto a particulate should be quantitatively and easily removed.

(d) Ease of operation; the sampling system should be relatively simple to construct and to operate.

(e) Applicability; the sampler should be usable for both raw water and treated water.

The study protocol, as shown in Fig. 2, was designed to select the particulate that had the highest probability of retaining or releasing cysts under specified experimental conditions and to eliminate those particulates that had a lower probability. In addition, experiments were designed to determine the effects of sampler design variables and water characteristics on the success of the sampling system.

ION-EXCHANGE CAPACITY STUDIES

Optimal pH for Cyst Retention on Each Resin. In order to determine the relative capacity of each resin to concentrate cysts from a liquid suspension, cyst retention was evaluated over a pH range. One ml of a cyst suspension containing between 5 and 50 $\times$ 10^4 cysts/ml was loaded onto anion and cation

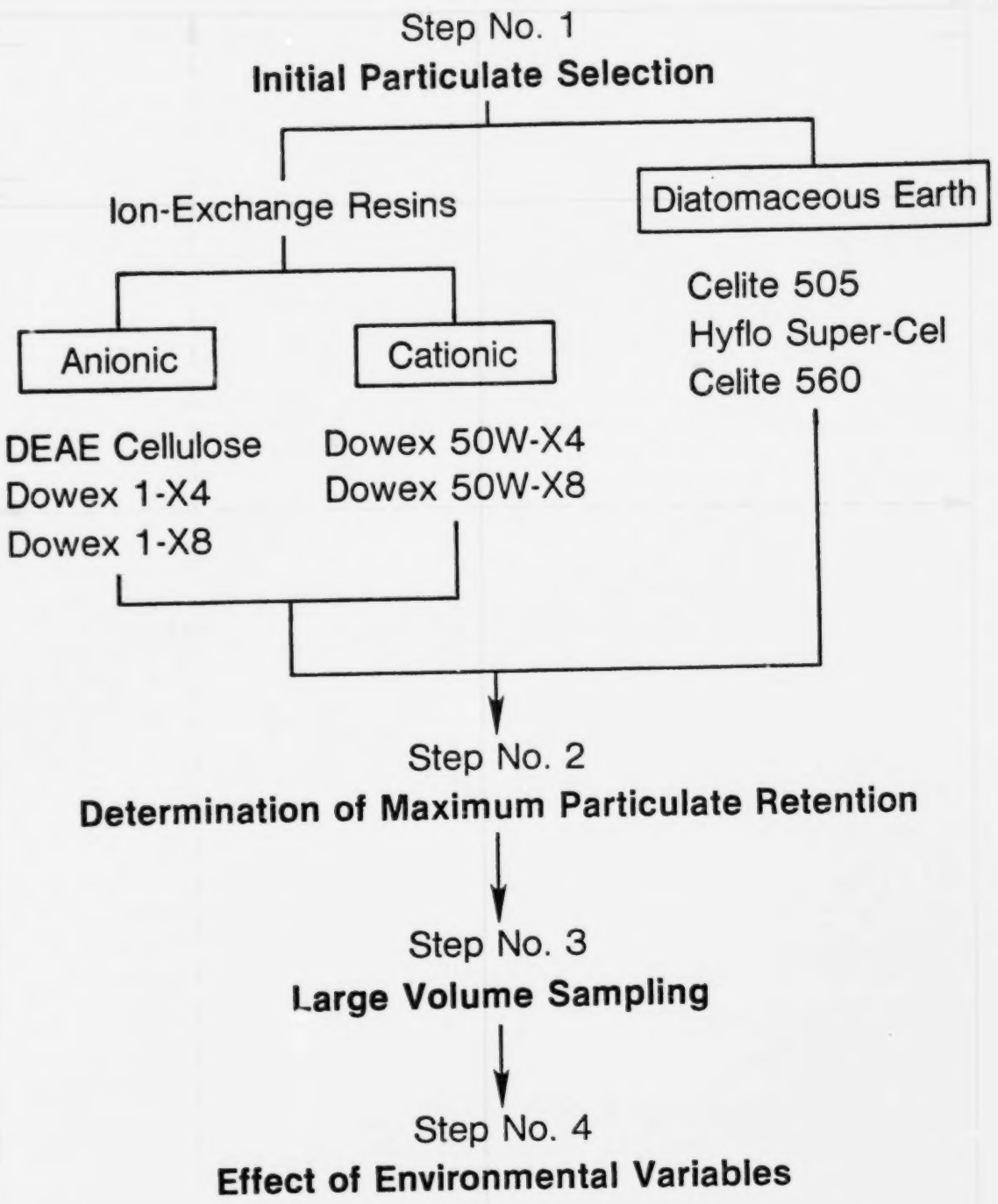


Figure 2. Cyst Sampling Study Protocol

exchange resins ranging in pH from 5 to 9. The cysts were mixed with the resins by gentle inversion for 10 min and then allowed to settle. The resin-liquid mixture was poured onto a 5 um membrane filter and washed with 10 ml deionized water containing 0.02% Tween 20. The exchange resin was carefully brushed from the filter surface and the filter was then vibrated in 10 ml deionized water containing 0.02% Tween 20 for 2 min. Following centrifugation, the cyst pellet was resuspended in 1 ml distilled water and counted.

Resin Capacity for Concentrating Cysts. Ten grams of each resin that had been washed in deionized water and ionically-charged were placed into a 22.0 cm X 1.5 cm (ID) plastic column and maintained in 25 ml of buffer prepared at the appropriate pH for maximum cyst retention. Each column was then loaded with a 1 ml suspension of deionized water containing various cyst concentrations in the range of 1.0×10^4 - 1.0×10^6 cysts/ml. The suspension was mixed by gentle inversion for 10 min; free cysts were concentrated onto 5 um membrane filters and enumerated as described above. The number of cysts retained was determined as the difference between the number of cysts inoculated onto a column and the number of free cysts recovered in the spent solute.

In order to judge which ion-exchange resin(s) best retained cysts, the retention efficiency (number of cysts retained/number of cysts inoculated) and retention capacity (total available retention sites) were calculated from the data. The efficiency was determined by plotting the concentration of cysts retained as a function of the concentration of cysts inoculated onto a particular resin column. Slopes of the resulting lines were derived from linear regression lines (29) and compared at a 95% confidence level to determine the relative efficiency of each resin. The capacity of a particular resin was determined as a half saturation constant. Since the limiting factor in the overall capacity of an ion-exchange resin to retain a charged species is the number of available exchange sites (25), a maximum capacity should be achieved. Under such conditions, the exchange sites on the resin would become saturated and the retention will not increase with increasing input concentration. The retention of such a system can thus be described mathematically with saturation kinetics, similar to those used to describe the velocity of enzyme activities (25). In the case of cyst retention on ion-exchange resins, the saturation equation may be written as:

$$Cr = \frac{Cr_{max}\,[C_i]}{k_c + [C_i]} \qquad (5)$$

where Cr = concentration of cysts retained by a resin

Cr_{max} = theoretical maximum concentration of cysts retained

k_c = half-saturation constant; i.e., the concentration of cyst inoculated to yield 1/2 Cr_{max}

C_i = inoculum concentration

In order to determine the kinetic constants (Cr_{max} and k_c) the reciprocal of the above equation yielded a straight line with a slope of k_c/Cr_{max} and a y-intercept of $1/Cr_{max}$:

$$\frac{1}{Cr} = \frac{k_c}{Cr_{max}} \cdot \frac{1}{C_i} + \frac{1}{Cr_{max}} \qquad (6)$$

Therefore it was possible to compare the capacities of each ion-exchange resin based upon the k_c or half-saturation constant value.

CYST RECOVERY STUDIES

Giardia cysts retained on ion-exchange resins were eluted to determine the most efficient resin for sampling purposes. Since elution is a function of pH and ionic strength (25), each resin was evaluated over a pH range of 5 - 9 by eluting in 40 ml of buffer at a known pH and ionic strength. The resin was mixed in buffer for 2 min and free cysts were then concentrated on 5 um membrane filters. The elution efficiency of a particular resin was determined by loading columns with various cyst concentrations ranging from 1.0×10^3 - 1.0×10^6 cysts/ml and then determining the number of cysts recovered under optimum pH and ionic strength conditions.

DIATOMACEOUS EARTH STUDIES

Cyst Capture Determinations. A series of 20 liter water samples were inoculated with cysts to yield final concentrations ranging from 6 cysts/liter to 1.62×10^4 cysts/liter by serially diluting a stock solution of 3.28×10^5 cysts/ml and then adding 1.0 ml of diluent to 20 liters of water. Each dilution was prepared in triplicate and then passed through 40 gm of either Celite 505, Hyflo SuperCel or Celite 560 held in a sampler similar to that shown in Figure 3. All samples were filtered at a flow rate of 1.0 liter/min. The filtered eluant was collected, concentrated via membrane filtration, and counted to determine the number of cysts that passed through each diatomaceous earth filter.

Cyst Recovery Studies. Cysts concentrated on the surface of diatomaceous earth filters were removed by backwashing each filter with distilled water. Each filter was backwashed with 3000 ml of distilled water in 500 ml increments to determine the optimum backwash volume in 5 replicate trials. Following the determination of an optimum backwash volume, experiments were carried out to optimize backwash flow rate. Flow rates were varied between 1 and 4 liters/min (lpm) at 0.5 lpm intervals and the number of cysts recovered from each filter was compared.

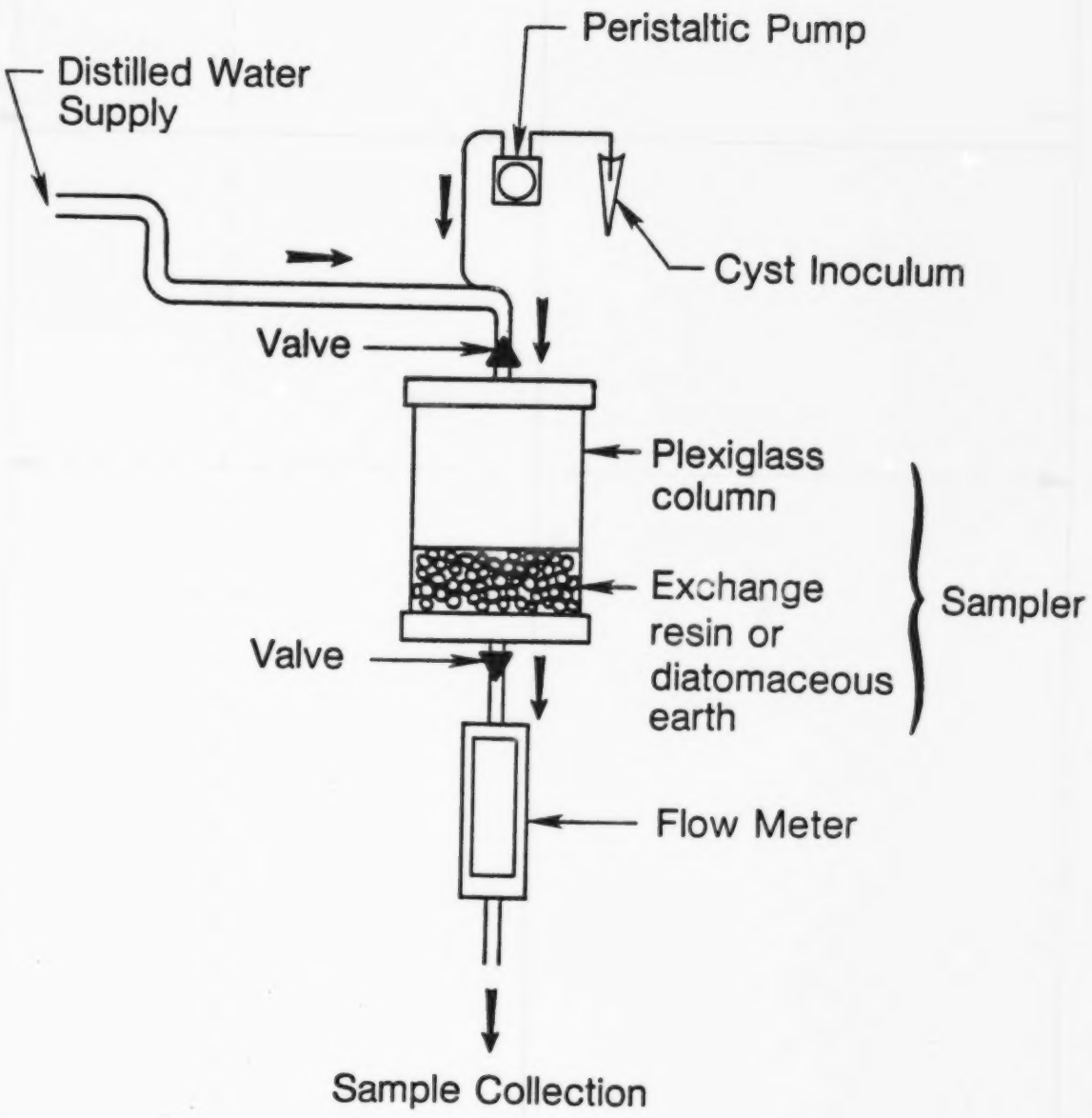


Figure 3. Laboratory Cyst Sampling Apparatus

LARGE VOLUME WATER SAMPLING

Based upon the results of the ion-exchange resin capacity and cyst recovery experiments, one ion-exchange resin and diatomaceous earth filter were selected to evaluate and compare under large volume sampling conditions.

Sampler Design. Bench-scale experiments were designed in such a manner that water volumes up to 40 liters could be sampled under controlled conditions of known sampling volume, flow rate and cyst inoculum concentration. The experimental design is shown in Figure 3. Water for experiments was supplied from a laboratory distilled water tap to the sampler through 12 mm inside diameter (I.D.) tygon tubing. Cysts were fed to the water line at a controlled rate with a variable speed peristaltic pump (Masterflex Pumps, Cole-Palmer Instruments Co., Chicago, Ill.) through 1 mm I.D. tygon tubing that was inserted into the water line with a 25 guage needle.

The sampler consisted of a plexiglass cylinder, 22 cm high with an I.D. of 10 cm and a 2 liter volume. Threaded caps containing stainless steel screens supporting a 15 um pore size silk screen were placed on each end. A rubber O-ring was placed between the caps and the sampler to seal the system. The inlet and outlet consisted of 12 mm I.D. galvanized pipe with cut-off valves. The sampling column was filled with 40 gm of particulate medium (ion-exchange resin or diatomaceous earth) and 1 liter deionized water before experimental sampling.

Cyst Recovery Efficiency Studies. A series of experiments were carried out to determine the relative efficiency of a particular sampling system. While the total volume of distilled water (pH = 7.0) sampled remained constant at 40 liters from experiment to experiment, the sampling medium, flow rate, and cyst concentration were varied. Flow rates were varied from 1 liter/min to 4 liters/min and cyst concentrations ranged from 1×10^3 to 1×10^5 cysts/liter of sample water. Following filtration, excess water in the sampler was drained and cysts were recovered from the particulate. In the case of ion-exchange filtration, the resins were removed from the plexiglass column by washing into 100 ml of eluant (pH 7.0, total ionic strength = 1.0M) containing 0.02% Tween 20, vibrating for 2 min, and concentrating free cysts on 5.0 um membrane filters. Cysts were recovered from diatomaceous earth filters by back washing the filter with 2 liters distilled water at a flow rate of 2 liters/min. Free cysts in the backwash were concentrated on 5.0 um membrane filters for quantification.

The sampling efficiency of each filter medium was determined by comparing the number of cysts recovered with the initial cyst concentration of the water sample. A series of comparisons were made for each flow rate and at each inoculum concentration. The effect of flow rate or initial cyst concentration on the outcome of the experiment was determined through analysis of variance (29). Following data analysis and selection of the most efficient sampling system, a series of experiments were carried out to determine a minimum detection limit. Forty-liter water samples were run through the sampling system with progressively lower cyst concentrations until no cysts were re-

covered from the sampler. That concentration point was considered to be the minimum detection limit under laboratory conditions.

Determination of the Effects of Water Parameters on Sampling Efficiency.

Laboratory experiments to this point had indicated that diatomaceous earth filters had best met the first four criteria outlined in the study protocol. Therefore, it was selected for the final phase of the study. The parameters that were evaluated included pH and turbidity. A series of 40 liter samples were run through the column at varying pH and turbidity values. Turbidity values ranged from 25 to 0 formazin turbidity units (FTU) while pH was varied between 5.0-9.0. A turbidity of , FTU was selected as the upper limit since most natural surface waters rarely exceed this value (1). Water samples for these experiments were collected from the Little Miami River in sufficient quantity (approx. 80 liters) to run a series of 12 experiments. Turbidity values were adjusted by the dilution of the river water samples with distilled water, while pH adjustments were made by the step-wise addition of acid (0.1 N HCl) or base (1.0 N NaOH).

SECTION 6

RESULTS AND DISCUSSION

STANDARDIZATION OF TECHNIQUES

In order to meet the objectives of this study, the relative ability of each resin to retain and/or release cysts was determined in quantitative terms (concentration or number retained, % retention, or % release). Therefore, it was important to develop standardized laboratory techniques and to know the variability of each technique. Methods of cyst concentration and cyst counting were standardized and the variability associated with each procedure was measured. Cysts collected from a particular resin were concentrated onto membrane filters (5 um), recovered in 0.02% Tween 20 and centrifuged for counting. Counts were carried out with a hemacytometer.

Standardization of Hemacytometer Counts. Preliminary experiments with stock cultures of G. muris cysts indicated that a 10 ul sample volume filled each counting well of the hemacytometer and helped ensure repeatable cell counts when multiple portions of a sample were examined. While the actual volume of sample in a well may have varied slightly with coverslip placement, the use of a 10 ul pipette ensured even distribution in a well. This method facilitated cyst dispersal within each well and prevented accidental overfill which resulted in variability between counts (2).

The variability associated with the count of a sample was determined by making fifteen replicate counts of a stock culture diluted to approximately 3.0×10^4 cysts/ml with distilled water. The results are shown in Table 1. Each millimeter square counting area contained between 20 and 40 cysts with a mean of 27.53 and a standard deviation of 5.14. Therefore, the coefficient of variation (CV) associated with the counting procedure was 0.19. Substitution of this value into equation (3) with a 95% confidence level ($k = 1.645$) indicated that when at least 10 millimeter square areas were counted, the counting error (% E) was 10% or less. Therefore, samples were manipulated (diluted or concentrated) when possible so that each counting area contained 20-40 cysts.

Membrane Filter Concentration of Cysts. Observations of cysts concentrated on the surface of membrane filters and subsequently recovered in distilled water indicated that a large portion of the cysts recovered were clumped together to such an extent that individual cysts could not be counted. Therefore cysts concentrated on 5 um membrane filters were resuspended in 0.02%

TABLE 1. VARIABILITY OF CYST COUNTS WITH A HEMACYTOMETER

Replicate Count	Cysts/Square mm Counting Area
1	22
2	30
3	34
4	28
5	28
6	34
7	33
8	24
9	25
10	21
11	22
12	34
13	19
14	31
15	28
$\bar{X}$	27.53
s	5.14

Tween 20 to disperse individual cysts. In order to determine the recovery efficiency of this procedure, 20 one-liter water samples were inoculated with cysts to yield final concentrations ranging from 1.0×10^5 to 1.0×10^6 cysts/ml, concentrated via membrane filtration, resuspended in 10 ml 0.02% Tween 20, and centrifuged for counting. The concentration of cysts recovered was compared to the inoculum concentration to determine the recovery efficiency. The results of the experiments are given in Table 2. Recoveries ranged from 40% to 119% with a mean recovery of 84.1% over the range of concentrations examined. Calculation of a 95% confidence interval around this mean recovery using equation 4 indicated that between 74.5% and 93.7% of the cysts in a sample could be recovered from the surface of membrane filters 95% of the time.

EVALUATION OF ION-EXCHANGE RESINS

Determination of Optimum Retention pH. The first step in the evaluation of ion-exchange resins as cyst sampling media was to determine the optimum retention pH. Using cyst concentrations between 5×10^4 and 5×10^5 cysts/ml, each resin-type (DEAE cellulose, anionic, and cationic) was evaluated over a pH range of 5.0 to 9.0. The results are given in Tables 3a and b. Retention of cysts on DEAE cellulose beads was unaffected by pH, whereas 70-80% of the inoculum were retained on anion exchangers at a pH of 8.0 or greater (Table 3a). Cation exchangers retained cysts most efficiently (35-45%) at a pH of 6.5 or lower (Table 3b). Ionic strength of the buffer was maintained at a low level ($pI \leq 0.1$) to permit maximum retention without interference of competing ions.

Cyst Adsorption Onto Ion-Exchange Resins. The ability of each ion-exchange resin to concentrate cysts from water samples was determined by diluting stock cyst cultures in 100 ml of buffer adjusted to the appropriate pH for each resin (pH of 8.0-9.0 for anion exchanges including DEAE cellulose and pH of 5.0-6.0 for cation exchangers) to yield final concentrations between $10^4 - 10^5$ cysts/ml. After passing each sample through a resin, the number of cysts in the eluant was counted. The results are shown in Table 4a and 4b. The retention of cysts on each resin was plotted as a function of the inoculum concentration as shown in Figure 4. The relative retention efficiency of each resin was determined from the slopes of regression lines (29), while the retention capacity was estimated as a half-saturation constant (k_c) as described in equation (6). The results are given in Table 5. Comparison of the slopes at a 95% confidence level using Duncan's multiple range test (29) indicated that DEAE cellulose, Dowex 1-X4 and Dowex 50W-X8 resins were significantly more efficient at retaining cysts than were the other resins. Comparison of half-saturation constants (k_c) also indicated that these three resins had a greater capacity for cyst adsorption, yet they were not significantly different from each other ($p > 0.05$) when compared at a 95% confidence level.

TABLE 2. RECOVERY OF CYSTS CONCENTRATED ON 5 um MEMBRANE FILTERS

Sample No.	Inoculum Concentration (Cysts/ml X 10^6)	Cysts Recovered (Cysts/ml X 10^6)	% Recovered
1	0.20	0.20	100
2	0.50	0.20	40
3	0.70	0.50	71
4	0.70	0.60	86
5	0.80	0.70	88
6	1.10	1.20	109
7	1.20	1.10	92
8	1.20	1.20	100
9	1.50	1.00	67
10	1.50	1.00	67
11	2.40	1.80	75
12	2.60	1.90	73
13	3.00	1.80	60
14	3.00	2.20	73
15	3.20	3.50	109
16	3.20	3.80	119
17	3.40	3.70	109
18	3.80	2.40	63
19	3.80	2.50	66
20	3.80	4.40	116
$\bar{X}$	2.08	1.78	84.1

TABLE 3a. EFFECT OF pH ON CYST RETENTION BY ANION EXCHANGERS

pH	INOCULUM CONCENTRATION (10^4 cysts/ml)	CYSTS RETAINED ON: DEAE CELLULOSE Concentration Retained (10^4 cysts/ml)	DEAE CELLULOSE % Retained	DOWEX 1-X4 Concentration Retained (10^4 cysts/ml)	DOWEX 1-X4 % Retained	DOWEX 1-X8 Concentration Retained (10^4 cysts/ml)	DOWEX 1-X8 % Retained
5	5	5	100	0.38	7.5	0.35	7.0
	10	10	100	0.80	8.0	0.85	8.5
	50	50	100	4.50	9.0	3.00	6.0
6	5	5	100	0.60	12	0.50	10
	10	10	100	1.00	10	1.00	10
	50	50	100	3.50	7	6.00	12
7	5	5	100	2.40	48	2.10	42
	10	10	100	4.40	44	4.60	46
	50	50	100	22.0	44	23.0	46
8	5	5	100	3.65	73	3.50	70
	10	10	100	7.00	70	7.30	73
	50	50	100	37.5	75	37.5	75
9	5	5	100	3.75	75	3.75	75
	10	10	100	8.00	80	8.40	84
	50	50	100	43.0	86	42.0	84

TABLE 3b. EFFECT OF pH ON CYST RETENTION BY CATION EXCHANGERS

pH	INOCULUM CONCENTRATION (10^4 cysts/ml)	CYSTS RETAINED ON: DOWEX 50W-X4 Concentration Retained (10^4 cysts/ml)	DOWEX 50W-X4 % Retained	DOWEX 50W-X8 Concentration Retained (10^4 cysts/ml)	DOWEX 50W-X8 % Retained
5	5	2.15	43	2.00	40
	10	3.50	35	4.20	42
	50	23.0	46	23.5	47
6	5	1.75	35	1.70	34
	10	3.70	37	3.80	38
	50	15.5	31	15.0	30
7	5	1.00	20	0.95	19
	10	1.80	18	1.70	17
	50	10.0	20	7.50	15
8	5	0.25	2	0.20	4
	10	0.60	6	0.40	4
	50	3.50	7	2.50	5
9	5	0.05	1	0.10	2
	10	0.20	2	0.20	2
	50	2.50	0.5	1.00	2

TABLE 4a. RETENTION OF G. MURIS CYSTS BY ANION EXCHANGE RESINS

SAMPLE NO.	ION EXCHANGE RESIN:					
	DEAE CELLULOSE		DOWEX 1-X4		DOWEX 1-X8	
	Cyst Inoculum [1.]	Conc. Retained [1.]	Cyst Inoculum	Conc. Retained	Cyst Inoculum	Conc. Retained
1	12.6	12.6	12.6	8.5	10.7	5.3
2	12.6	12.6	12.6	9.3	10.7	4.5
3	12.6	12.6	12.6	9.0	10.7	6.6
4	18.4	18.4	17.6	14.5	20.2	12.6
5	18.4	18.4	17.6	14.5	20.2	12.1
6	18.4	18.4	17.6	15.7	20.2	11.8
7	30.0	30.0	26.9	24.5	34.6	21.9
8	30.0	30.0	26.9	25.9	34.6	18.4
9	30.0	30.0	26.9	25.0	34.6	22.5
10	45.2	45.2	41.6	39.2	40.0	29.8
11	45.2	45.2	41.6	39.1	40.0	25.7
12	45.2	45.2	41.6	39.4	40.0	26.2
13	55.0	55.0	54.7	50.8	57.9	37.1
14	55.0	55.0	54.7	50.8	57.9	35.7
15	55.0	55.0	54.7	52.5	57.9	42.6

1. All concentrations are multiplied by 1 X 10^4 cysts/ml

TABLE 4b. RETENTION OF G. MURIS CYSTS BY CATION EXCHANGE RESINS

SAMPLE NO.	ION EXCHANGE RESIN:			
	DOWEX 50W - X4		DOWEX 50W - X8	
	Cyst Inoculum [1.]	Conc. Retained [1.]	Cyst Inoculum	Conc. Retained
1	5.8	4.6	8.6	8.2
2	5.8	4.7	8.6	7.6
3	5.8	5.4	8.6	7.5
4	21.6	17.0	21.3	20.7
5	21.6	18.8	21.3	20.0
6	21.6	16.1	21.3	20.4
7	39.0	28.0	34.7	31.2
8	39.0	31.4	34.7	30.2
9	39.0	31.5	34.7	27.0
10	52.8	43.1	51.9	49.0
11	52.8	40.1	51.9	49.3
12	52.8	37.9	51.9	48.9
13	65.3	50.1	70.5	68.8
14	65.3	53.3	70.5	67.0
15	65.3	47.0	70.5	67.2

1. All concentrations are multiplied by 1 X 10^4 cysts/ml.

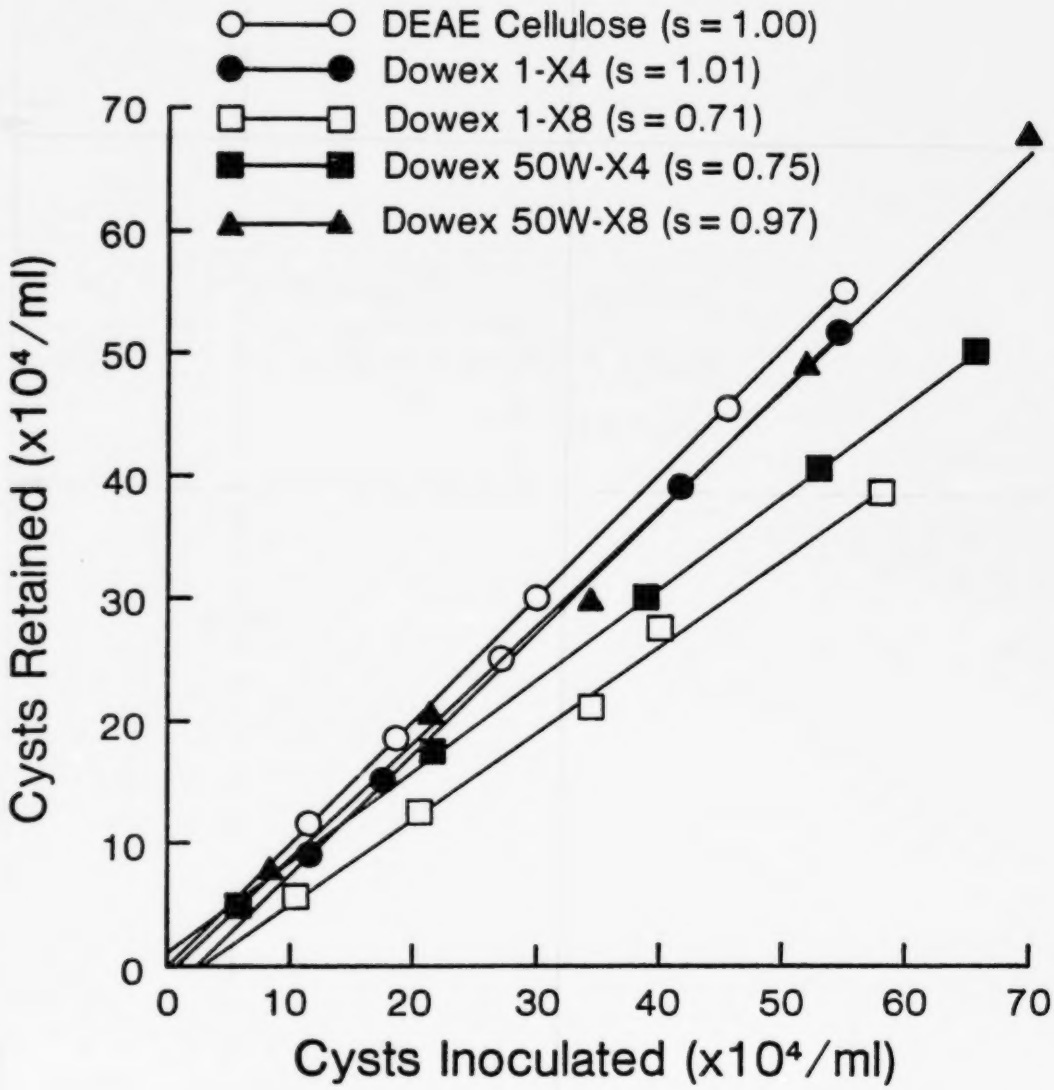


Figure 4. Adsorption of Cysts to Ion-Exchange Resins

(Each point is the mean of 3 replicates)

TABLE 5. ADSORPTION CHARACTERISTICS OF *GIARDIA* CYSTS ONTO ION-EXCHANGE RESINS

Ion-Exchange Resin	Retention Efficiency[1]	Retention Capacity[2] (k_c) (Cysts retained/gm resin)
DEAE Cellulose	1.00[3]	——
Dowex 1 - X4	1.01	2.5×10^6[3]
Dowex 1 - X8	0.71	1.1×10^5
Dowex 50W - X4	0.75	7.8×10^4
Dowex 50W - X8	0.97	5.5×10^6

(1) Retention efficiency was calculated as the slopes of regression lines of concentration of cysts retained vs. cyst inoculum concentration.

(2) Retention capacity was estimated as the k_c or half-saturation constant.

(3) All values are the mean of three replicate analyses.

Recovery of Cysts from Ion-Exchange Resins. Following the study protocol outlined in Figure 2, Dowex 1-X8 and Dowex 50W-X4 were eliminated from further study, while DEAE cellulose, Dowex 1-X4, and Dowex 50W-X8 were evaluated for cyst recovery. In order to determine the optimum cyst elution conditions for each resin a series of experiments was carried out to assess the effects of varying pH and buffer ionic strength (pI) on cyst recovery. A stock solution of G. muris cysts was diluted into 100 ml distilled water (pH of 8.0-9.0 for anionic resins and pH of 5.0 for cationic resins) with a pI of < 0.1 to yield a final concentration of 1.0×10^4 to 1.0×10^5 cysts/ml. Following cyst adsorption each resin was eluted with 40 ml of buffers ranging in pH from 5 to 9 and pI from 0.1 to 1.0. The results are given in Table 6. The data in Table 6 was normalized by calculating the percent recovery in each experiment and then plotted as a function of the pH as shown in Figure 5. The effects of pH and/or pI on the outcome of each experiment was determined through analysis of variance using ANOVA (29). Mean percent recoveries of each replicate set of experiments were calculated and compared at a 95% confidence level using Duncan's Multiple Range Test for variability (29). The percentage of cysts recovered from Dowex 1-X4 resins was not affected by pH, pI, or any combinations of pH and pI. However, recovery of cysts from Dowex 50W-X8 was pH dependent ($p = 0.006$) in that recoveries at buffer pH's of 7 or greater were significantly higher than at a buffer pH of 5.

Following pH and ionic strength adjustment, adsorbed cysts were eluted from each exchange resin. Cyst recovery was evaluated over an inoculum range of 1.0×10^3 to 1.0×10^6 cysts/ml and the number of cysts recovered from each resin is listed in Table 7. The average concentration of cysts recovered was calculated from the data in Table 7 and plotted as a function of inoculum concentration as shown in Figure 6. Lines were fitted to the data through linear regression analysis and the resulting slopes were compared at a 95% confidence level (29). A greater fraction of the inoculated cysts were consistently recovered from the Dowex 1-X4 resin over the entire concentration range tested.

Since both an anion exchanger (Dowex 1-X4) and a cation exchanger (Dowex 50W-X8) demonstrated a similar ability to concentrate cysts from water samples, the selection of a single ion-exchange resin for further study was unclear. It appeared that ion-exchange did not account for the observed retention and recovery rates. However, several observations made during the study indicated that charge attraction was a likely mechanism of attraction. These observations included:

(1) As the pH was increased from pH 5 to pH 9, the concentration of cysts adsorbed onto anion-exchange resins increased.
(2) As the pH was decreased from pH 9 to pH 5, the concentration of cysts adsorbed onto cation-exchange resins also increased.
(3) As the pH was increased from pH 5 to pH 9, the concentration of cysts recovered from cation-exchange resins increased.

While these observations appear to be contradictory and opposite to what would be expected to occur in normal ion-exchange processes, two additional facts help to explain the observed results. First, strong anion and cation exchangers were initially selected for the study so that they would be useful under a variety of environmental conditions. Strong ion-exchangers are characterized by the fact that the charged group attached to the resin surface is

TABLE 6. THE EFFECT OF BUFFER pH AND IONIC STRENGTH ON CYST RECOVERY FROM ION-EXCHANGE RESINS

pH	DEAE CELLULOSE pI 0.1 Inoculum conc (1) / Eluted conc		DEAE CELLULOSE pI 0.5 Inoculum Conc / Eluted conc		DEAE CELLULOSE pI 1.0 Inoculum Conc / Eluted conc		DOWEX 1-X4 pI 0.1 Inoculum Conc / Eluted conc		DOWEX 1-X4 pI 0.5 Inoculum Conc / Eluted conc		DOWEX 1-X4 pI 1.0 Inoculum Conc / Eluted conc		DOWEX 50W-X8 pI 0.1 Inoculum Conc / Eluted conc		DOWEX 50W-X8 pI 0.5 Inoculum Conc / Eluted conc		DOWEX 50W-X8 pI 1.0 Inoculum Conc / Eluted conc	
5	33	0	28	0	31.9	0	33	24.4	28	15.4	31.9	29.3	33	2.3	28	1.7	31.9	2.4
	28.3	0	27.8	0	29.2	0	28.3	22.6	27.8	20.8	29.2	24.8	28.3	1.7	27.8	2.9	29.2	5.7
	28.1	0	30	0	29.4	0	28.1	24.2	30.0	24.0	29.4	17.6	28.1	5.8	30.0	5.4	29.4	7.0
7	30.5	0	43.6	0	37.5	0	30.5	18.3	43.6	34.9	37.5	41.2	30.5	16.7	43.6	28.2	37.5	10.8
	32.2	0	31.4	0	35.7	0	32.2	23.2	31.4	18.8	35.7	35.7	32.2	11.4	31.4	12.4	35.7	16.3
	32.8	0	30.6	0	30.0	0	32.8	18.7	30.6	25.1	30.0	29.7	32.8	9.9	30.6	18.7	30.0	15.1
9	26.8	0	29.9	0	34.4	0	26.8	17.4	29.9	14.9	34.4	24.4	26.8	3.6	29.9	8.0	34.4	14.2
	29.6	0	33.1	0	26.2	0	29.6	21.9	33.1	27.1	26.2	16.2	29.6	11.8	33.1	15.1	26.2	10.5
	38.0	0	34.3	0	28.6	0	38.0	29.3	34.3	19.6	28.6	22.9	38.0	9.8	34.3	17.5	28.6	6.5

(1) All concentrations are multiplied by 1 X 10^4 cysts/ml.

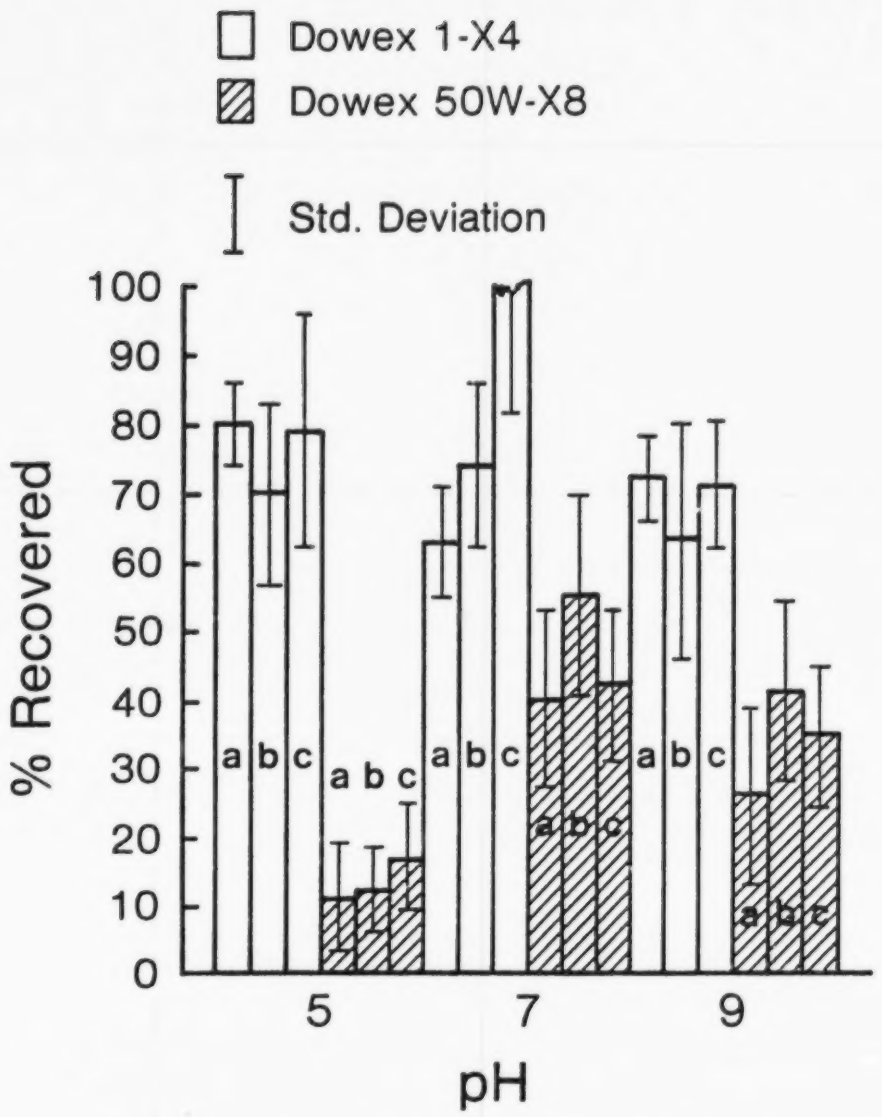


Figure 5. The Effect of pH and Ionic Strength (pI) on Cysts Recovered from Ion-Exchange Resins

(a. pI = 0.1, b. pI = 0.5 and c. pI = 1.0)

TABLE 7. RECOVERY OF G. MURIS FROM ION-EXCHANGE RESINS

Experiment No.	ION-EXCHANGE RESIN			
	DOWEX 1-X4		DOWEX 50W - X8	
	Inoculum Conc. (cysts/ml)	Conc. of Cysts Recovered (cysts/ml)	Inoculum Conc. (cysts/ml)	Conc. of Cysts Recovered (cysts/ml)
1	5.0×10^3	2.1×10^2	5.0×10^3	5.0×10^3
2	5.0×10^3	2.0×10^3	5.0×10^3	1.8×10^3
3	5.0×10^3	3.0×10^3	5.0×10^3	5.7×10^2
4	5.0×10^4	4.2×10^4	8.0×10^4	2.0×10^4
5	5.0×10^4	3.0×10^4	8.0×10^4	1.0×10^4
6	5.0×10^4	3.0×10^4	8.0×10^4	8.9×10^3
7	3.5×10^5	2.9×10^5	2.0×10^5	7.5×10^4
8	3.5×10^5	2.0×10^5	2.0×10^5	7.0×10^4
9	3.5×10^5	1.1×10^5	2.0×10^5	6.0×10^4
10	5.0×10^6	1.5×10^6	2.0×10^6	6.0×10^4
11	5.0×10^6	1.0×10^6	2.0×10^6	6.0×10^4
12	5.0×10^6	9.1×10^5	2.0×10^6	7.0×10^4

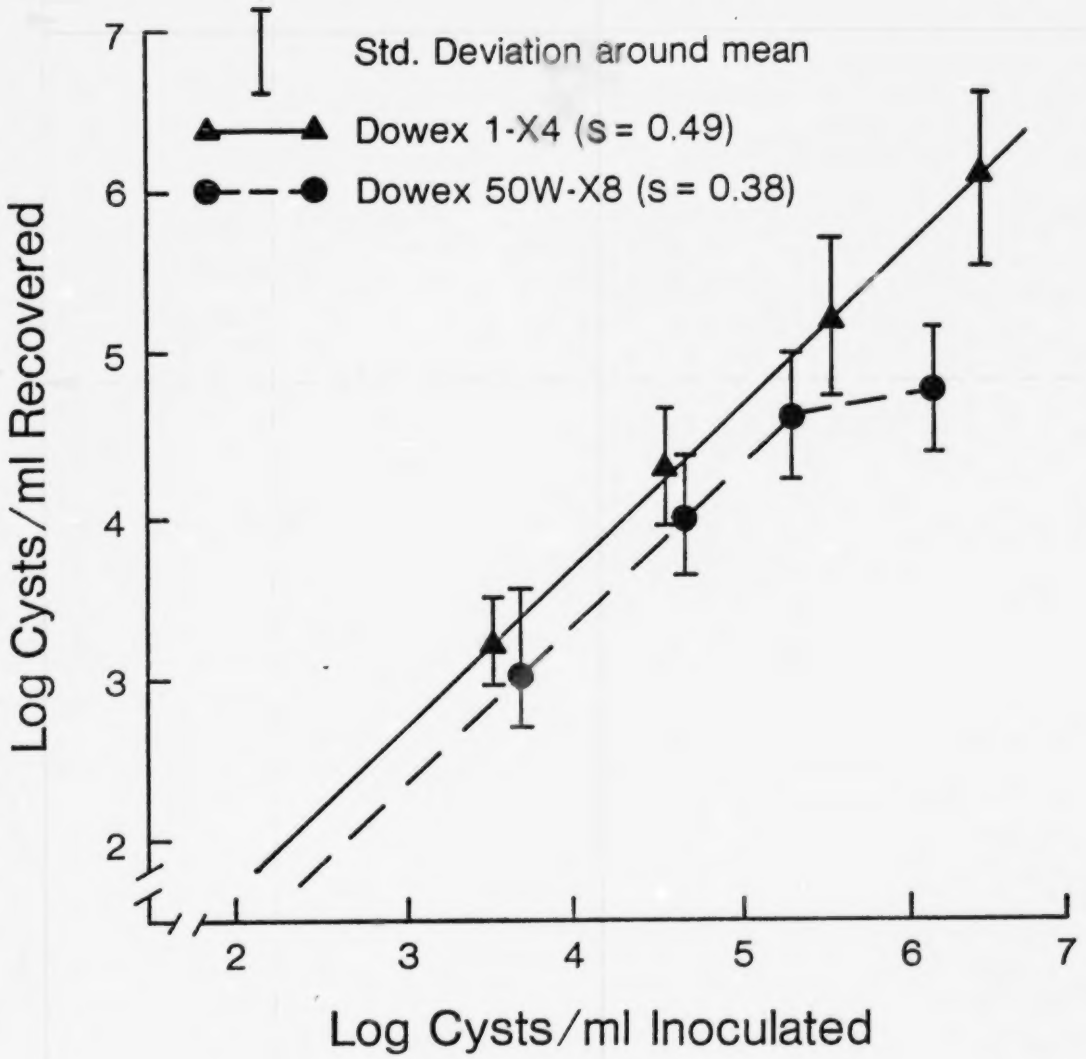


Figure 6. Desorption of Cyst from Ion-Exchange Resins

(Each point is the mean of 3 replicates)

either a strong acid or strong base. As can be seen in Figure 7, the capacity of neither a strong anion exchanger (line a) nor a strong cation exchanger (line b) would have been significantly altered within the pH range used in this study. Therefore, the observed differences in cyst retention and/or release by the resins as a function of pH were due to changes in the charge density surrounding the cysts. Since cysts of Giardia have been shown to be negatively charged at a pH of 5.5 and to increase the electronegativity as the pH rises to 8.0 (18), it appeared that cysts were attracted to the anion exchangers as a result of increased charge attraction with increasing pH. In low pH buffers (pH 5-6), the negative charge density was decreased and the cysts were attracted to the cation exchanger. This appeared to be the case in that as the pH was raised from 5 to 9, a higher concentration of cysts was recovered from the cation exchanger.

An alternative hypothesis to ion-exchange is that the cysts were adsorbed to the resin surfaces as a result of electrostatic attractive forces and not through ion-displacement mechanisms. Such an hypothesis would be supported by the differences observed in cyst adsorption by resins differing in cross-linkage. The effect of crosslinkage on the net surface charge of an ion-exchange resin is illustrated in Figure 8. As can be seen, the amount of cross-linking generally increases the net surface charge of an exchange resin. The data in Table 5 indicated that cyst retention efficiency by a particular resin was dependent on the amount of cross-linking of that resin. Electrostatic attractive forces occur when surfaces bear similar charges but have different surface potentials (4). In the case of cyst adsorption on anion-exchange resins, the highest rate of adsorption was observed with the lower cross-linked resin at a pH at which the cyst was most charged. Just the opposite was observed with cation exchangers. In both cases, the surface charge potentials between the cysts and resins were maximum under the experimental conditions used. Such an attraction has been observed between blood erythrocytes and DEAE cellulose beads (19).

The selection of an ion-exchange for further study was based upon cyst retention and recovery data. While Dowex 1-X4 and Dowex 50W - X8 appeared similar, DEAE Cellulose was eliminated on the basis that no cysts could be recovered from the resin under the experimental conditions used. Therefore, the selection of an ion-exchange resin for further study was based on the difference in cysts recoveries from the anion exchanger, Dowex 1-X4 and the cation exchanger, Dowex 50W-X8. As can be seen in Figure 6, a greater fraction of the inoculated cysts were recovered from the anion exchanger over the concentration ranged tested. Based on these data, Dowex 50W-X8 was eliminated from the study.

Microscopic Observations. While the data collected to this point had indicated that G. muris cysts could be efficiently concentrated from aqueous samples onto ion-exchange beads, less favorable results were observed when attempts were made to recover these cysts from the beads. Even under optimum conditions (Table 7) only half of the inoculated cysts could be recovered. Therefore to ensure that cysts were bound to the resin and not destroyed during the concentration procedures, microscopic observations of cyst-charged beads were made. Since Dowex 1-X4 had been selected as the ion-exchange resin

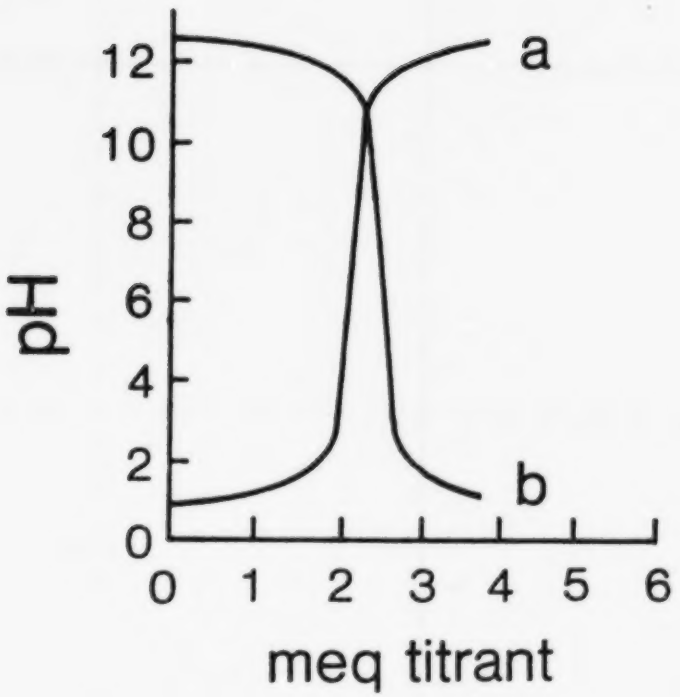
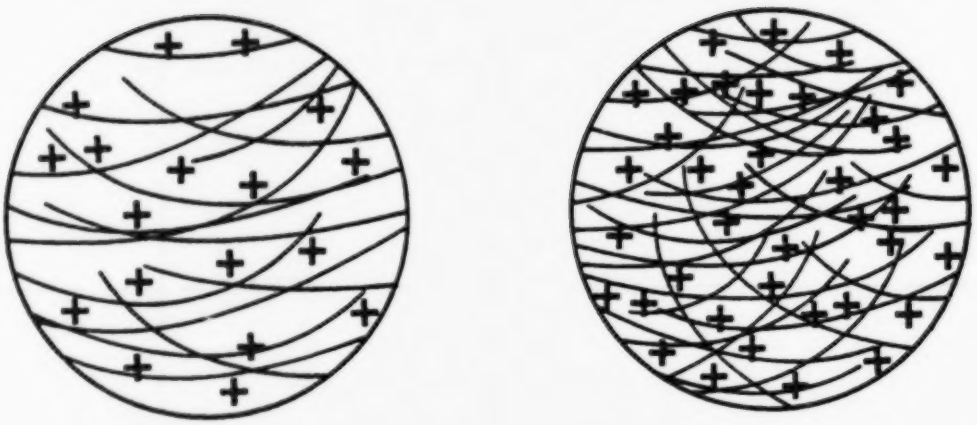


Figure 7. Titration of One Gram Ion-Exchanger in I M KCL (From Pharmacia Fine Chemicals AB, Uppsala, Sweden).

(a=strong anion exchanger)
(b=strong cation exchanger)



a. Low crosslinkage resin (e.g. 2%)

b. Highly crosslinked resin (e.g. 8%)

Figure 8. Effect of Crosslinkage on the Surface Charge of Ion-Exchange Resins
(From BioRad Laboratories, Richmond, California)

most likely to be adapted to field use, samples of this resin were removed from a column following inoculum with cysts and then microscopically observed. Light microscopic observations were made by carefully transferring a few beads into a hanging drop slide while scanning electron microscopic observations were made as previously described.

The light micrographs in Figures 9 and 10 indicated that numerous cysts were attached to the surface. At 400 X magnification (Figure 9) it appeared that cysts were attached along the edge of the beads, however, observations at 1000X magnification (Figure 10) indicated that the entire bead surface was covered with cysts. Scanning electron micrographs (Figures 11 and 12) also indicated that cysts were primarily attached to bead surfaces. Attempts to observe cysts attracted to the cation-exchange resin with scanning electron microscopy were unsuccessful in that no cysts were observed on bead surfaces.

EVALUATION OF DIATOMACEOUS EARTH

Cyst Retention. The capacity of each type of diatomaceous earth column to concentrate cysts from distilled water samples is given in Table 8. Over the inoculum concentration tested, no significant differences between the types of diatomaceous earth were noted. Recoveries ranged from 66 to 100% depending upon the inoculum concentration.

Cyst Recovery. Following the capture of cysts on the surface of the diatomaceous earth, each column was backwashed with distilled water to recover concentrated cysts. In the case of both Celite 505 and Hyflo-SuperCel, diatomaceous earth particles or "fines" were concentrated in the backwash to such an extent that cysts could not be observed in the final concentrate. Therefore, Celite 560 was used for further experimentation.

The recovery of cysts from diatomaceous earth (Celite 560) was optimized in relation to backwash flow rate and volume. Each of 5 replicate columns was inoculated with 8.1×10^3 cysts/ml by passing 100 ml of inoculum through each column. Backwashing was carried out at a flowrate of 1 lpm until 3000 ml of backwash was collected at 500 ml intervals. The concentration of cysts recovered per interval is given in Table 9. The concentration of cysts recovered at each 500 ml interval was converted to a percentage of the inoculum and values were then summed over the sampling range to determine the cumulative percent recovered at each sampling interval. This was then plotted as a function of backwash volume to determine the optimum volume as shown in Figure 13. Following the optimization of backwash volume, the best backwash flowrate was determined by backwashing 5 replicate columns with 2000 ml of distilled water at flowrates of 1 to 4 lpm at 0.5 lpm intervals. The concentration of cysts recovered in each backwash is given in Table 10. The recovered concentration was converted into a percentage of the inoculum concentration at each flowrate. Percentages for the 5 replicates were averaged and mean values were plotted as a function of flowrate as shown in Figure 14 in order to select the flowrate at which the greatest percentage of cysts were

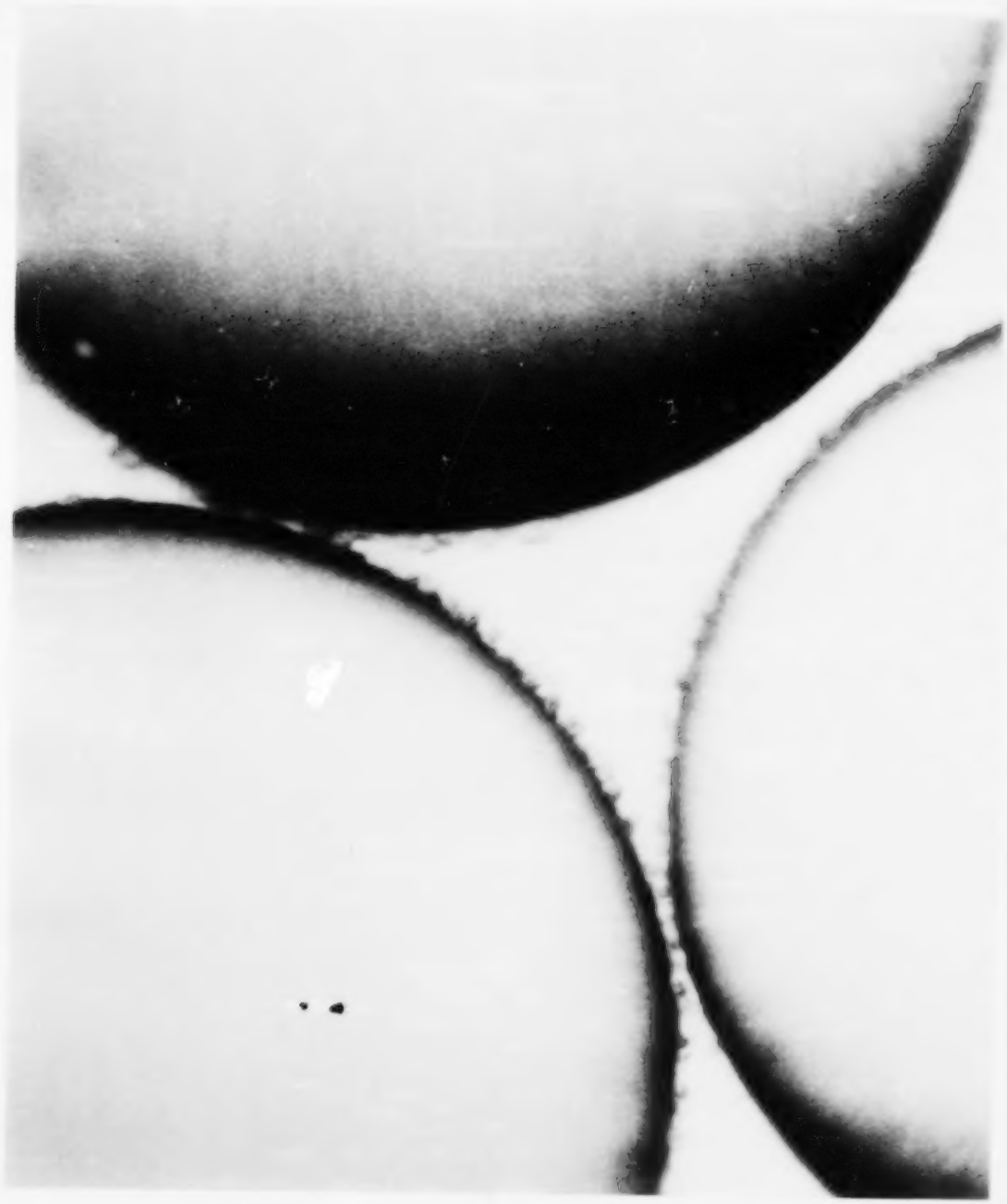


FIGURE 9. GIARDIA MURIS CYSTS ATTACHED TO DOWEX 1-X4 BEADS

FIGURE 10. GIARDIA MURIS CYSTS ATTRACTED TO THE SURFACE OF A DOWEX 1-X4 BEAD

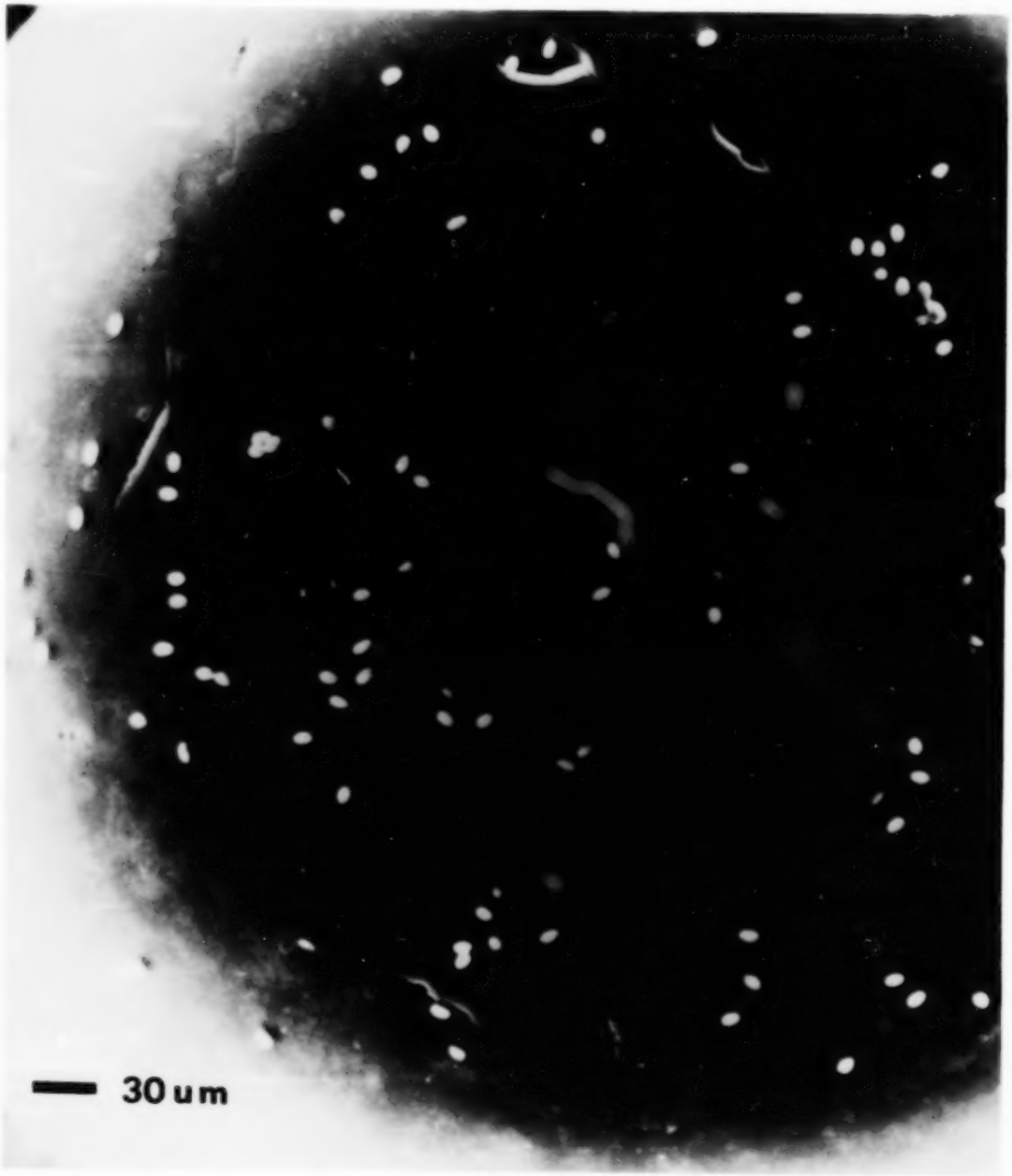


FIGURE 11. ELECTRON MICROGRAPH OF GIARDIA MURIS CYSTS ATTACHED TO BEAD SURFACES

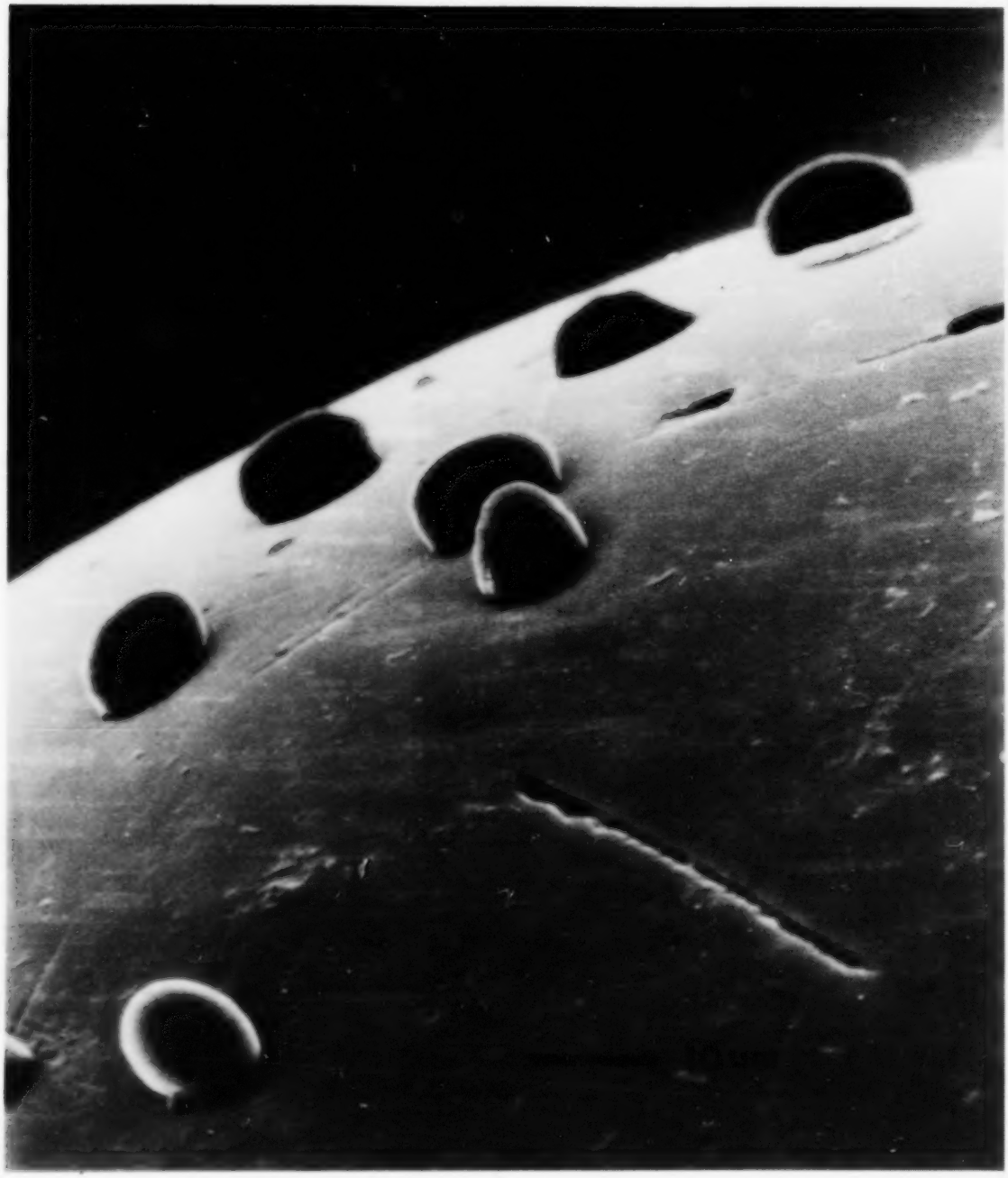


FIGURE 12. ELECTRON MICROGRAPH OF GIARDIA MURIS CYSTS CONCENTRATED ON DOWEX 1-X4 BEAD SURFACES

TABLE 8. ADSORPTION OF G. MURIS CYSTS ONTO DIATOMACEOUS EARTH FILTERS

Exp. No.	D.E. Type	No. Inoculated (cysts/liter)	No. Retained (cysts/liter)	% Retained
1	Celite 505	1.62×10^4	1.62×10^4	100
2		1.32×10^3	1.32×10^3	100
3		1.30×10^2	1.25×10^2	96.2
4		1.12×10^1	1.09×10^1	97.0
5		6.0	5.0	83.3
6		6.0	4.0	66.0
7	Hyflo	1.62×10^4	1.62×10^4	100
8		1.32×10^3	1.28×10^3	97
9		1.30×10^2	1.30×10^2	100
10		1.12×10^1	1.09×10^1	97
11		6.0	5.0	83.3
12		6.0	5.0	83.3
13	560	1.62×10^4	1.62×10^4	100
14		1.32×10^3	1.28×10^3	98
15		1.30×10^2	1.26×10^2	97
16		1.12×10^1	1.09×10^1	97
17		6.0	5.0	83.3
18		6.0	5.0	83.3

TABLE 9. THE EFFECT OF BACKWASH VOLUME ON CYST RECOVERY FROM DIATOMACEOUS EARTH COLUMNS

Replicate Column No.	Inoculum Conc. (cysts/ml X 10^3)	Recovery of Cysts at Each Sample Interval (cysts/ml X 10^3)					
		500ml	1000ml	1500ml	2000ml	2500ml	3000ml
1	8.1	1.4	0.9	0.9	0.42	0.12	0.05
2	8.1	0.5	2.6	1.4	0.12	0.18	0.08
3	8.1	1.7	1.7	0.5	0.14	0.14	0.06
4	8.1	0.4	2.8	1.4	0.50	0.20	0.08
5	8.1	1.7	1.9	0.8	0.40	0.40	0.30
$\bar{X}$	8.1	1.1	1.9	1.0	0.32	0.21	0.11

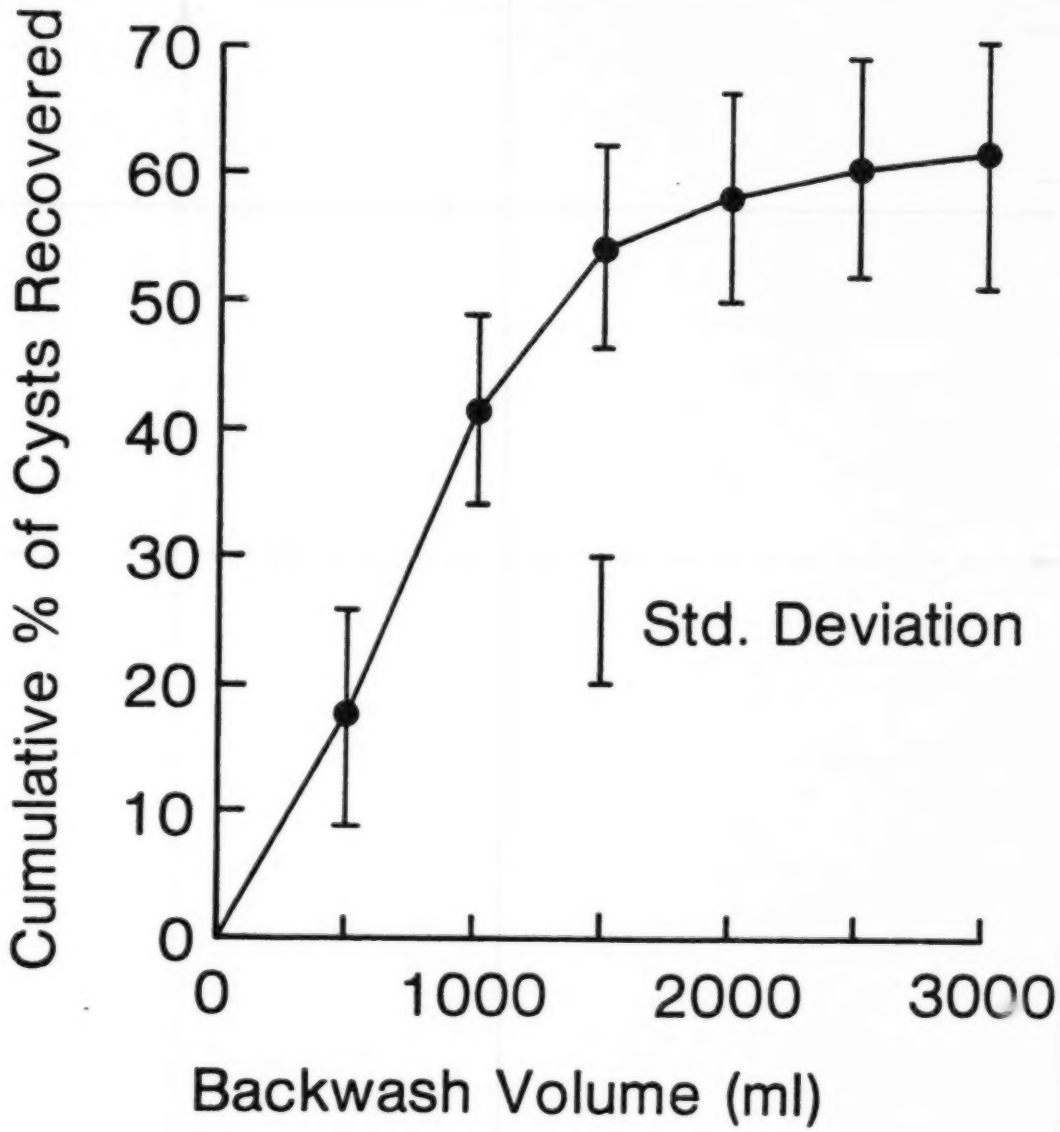


Figure 13. Recovery of Cysts from Diatomaceous Earth Filters at Various Backwash Volumes

(Each point is the mean of 5 replicates)

TABLE 10. THE EFFECT OF BACKWASH FLOWRATE ON CYST RECOVERY FROM DIATOMACEOUS EARTH COLUMNS

Backwash Flowrate (lpm)	Inoculum Conc. (cysts/ml 10^3)	Recovery of Cysts in Each Backwash (cysts/ml X 10^3)				
		1	2	3	4	5
1.0	50	15.5	13.5	7.0	20	19.2
1.5	75	46.5	51	32.2	30	40.5
2.0	40	30	24	24	23.2	18.8
2.5	85	50.6	52.7	49.3	54.4	46.2
3.0	25	14.5	10	16.2	15	16
3.5	40	20.8	16	20	24.4	24
4.0	50	25	15	22.5	18	30

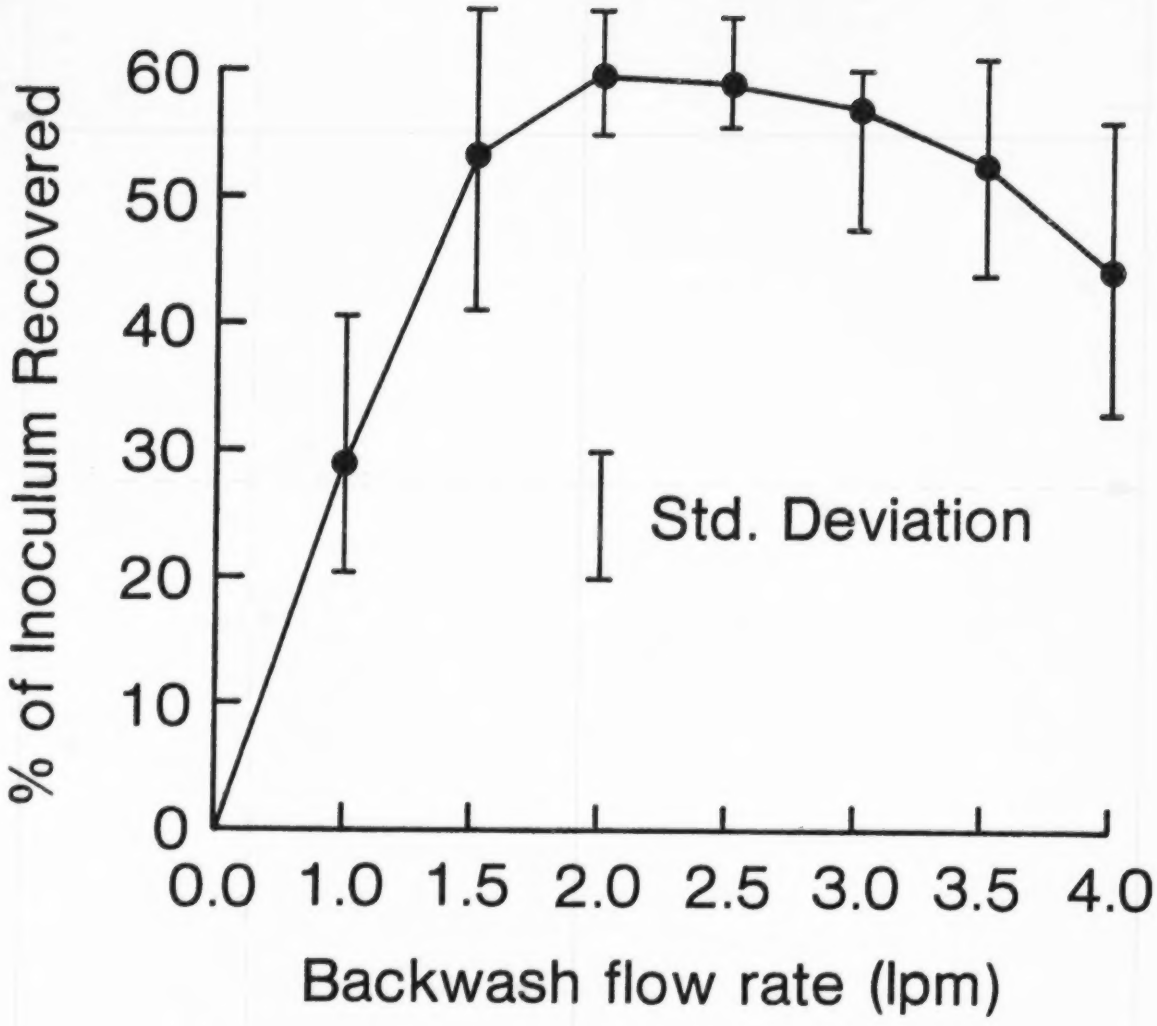


Figure 14. Determination of Optimum Backwash Flow Rate to Recover from Diatomaceous Earth

(Each point is the mean of 5 replicates)

recovered. The best recovery of cysts occurred when a column was backwashed with at least 2000 ml of distilled water at a flowrate of 2 lpm.

LARGE WATER VOLUME EXPERIMENTS

Following the study protocol outlined in Figure 2, the anion exchange resin, Dowex 1-X4 and diatomaceous earth (Celite 560) were selected for large volume sampling studies. The relative ability of each system to capture cysts from 40 liter distilled water samples and subsequently release concentrated cysts was evaluated under laboratory conditions similar to those depicted in Figure 3. The results of these experiments are given in Table 11. The results of each experiment were compared to select the sampling system most likely to recover cysts from water samples. The mean number of cysts recovered from either diatomaceous earth or Dowex 1-X4 over the entire experimental range was not significantly different ($p > 0.05$). When the number of cysts recovered was converted to a percentage of the inoculum (Table 11) and compared, no significant differences in sampling systems could be detected at a 95% confidence level. As a result, a "best" sampler could not be selected on the basis of the number of cysts recovered or the percentage of the inoculum recovered.

However, the critical judgement of the utility of a sampling system should be made under conditions similar to its intended use. Since water supplies will most likely contain low numbers of cysts per volume of water (12), sampling performance under such conditions was most important. The data in Table 11 was analyzed to distinguish any differences between the sampling ability of Dowex 1-X4 and diatomaceous earth at the lowest cyst inoculum concentrations used. When the log of the total number of cysts recovered in a particular experiment was plotted as a function of the log of the concentration of cysts inoculated into a sampling column, as shown in Figure 15, differences between diatomaceous earth and Dowex 1-4X were observed. When the rate of recovery (number of cysts recovered/cyst concentration in inoculum) measured by the slopes of the lines was compared, two patterns emerged. The recovery rate was significantly ($p = 0.05$) higher for Dowex 1-4X resins when compared to the rates observed with diatomaceous earth. However, it appeared that the number of cysts recovered from diatomaceous earth filters was not dependent upon the inoculum concentrations ($r = 0.56$), but upon some other variable in the sampling system. Analysis of variance (29) was carried out on the data to determine the best sampling conditions. The best recoveries of cysts at the lowest inoculum concentrations (1000-1500 cysts/liter) occurred when samples were filtered through diatomaceous earth columns at a flow rate of 1.0 liter/min.

To test the hypothesis that the probability of recovery cysts from dilute water samples was greater with the diatomaceous earth filter, a recovery ratio consisting of the total number of cysts recovered per inoculum concentration was computed and plotted as a function of the log of the inoculum concentration as shown in Figure 16. Two patterns emerged. At higher inoculum concentrations ($10^4 \times 10^5$ cysts/liter), there was little difference between the sampling systems; however, at ($1 \times 10^3 - 1.5 \times 10^3$ cysts/liter) inoculum

TABLE 11. RECOVERY OF GIARDIA CYSTS FROM FORTY LITER WATER SAMPLES

Sampling Medium	Sampling Flow Rate (liters/min)	No. of Cysts Inoculated into 40 liters ($X10^4$)	No. of Cysts Recovered ($X10^3$)	% Recovery
Diatomaceous Earth	1.0	1.18	2.50	21.1
		1.70	4.50	26.5
		1.88	5.00	26.6
		10.78	10.00	9.3
		11.10	8.00	7.2
		11.15	7.00	6.3
		27.50	17.00	6.2
		28.50	21.00	7.4
		30.00	20.00	6.7
Mean Recovery				13.0
	4.0	2.25	7.00	31.1
		2.35	3.00	12.8
		2.55	11.00	43.1
		22.25	10.00	4.5
		25.00	15.00	6.0
		25.00	21.00	8.4
		58.00	30.00	5.2
		69.50	47.00	6.8
		70.50	37.00	5.2
Mean Recovery				13.7

TABLE 11 (continued)

Dowex 1-X4	1.0	1.95	1.00	5.1
		2.75	1.00	3.6
		3.05	6.00	19.7
		19.00	14.00	7.4
		21.00	11.00	5.2
		23.50	14.00	5.9
		51.50	49.00	9.5
		63.50	52.00	8.2
		72.50	39.00	5.4
Mean Recovery				7.8
	4.0	3.25	2.00	6.1
		4.70	12.70	27.0
		5.00	4.50	9.0
		24.50	19.00	7.7
		24.50	32.00	13.1
		25.00	6.00	2.4
		72.50	89.00	12.3
		86.50	51.00	6.0
		95.00	130.00	13.7
Mean Recovery				10.8

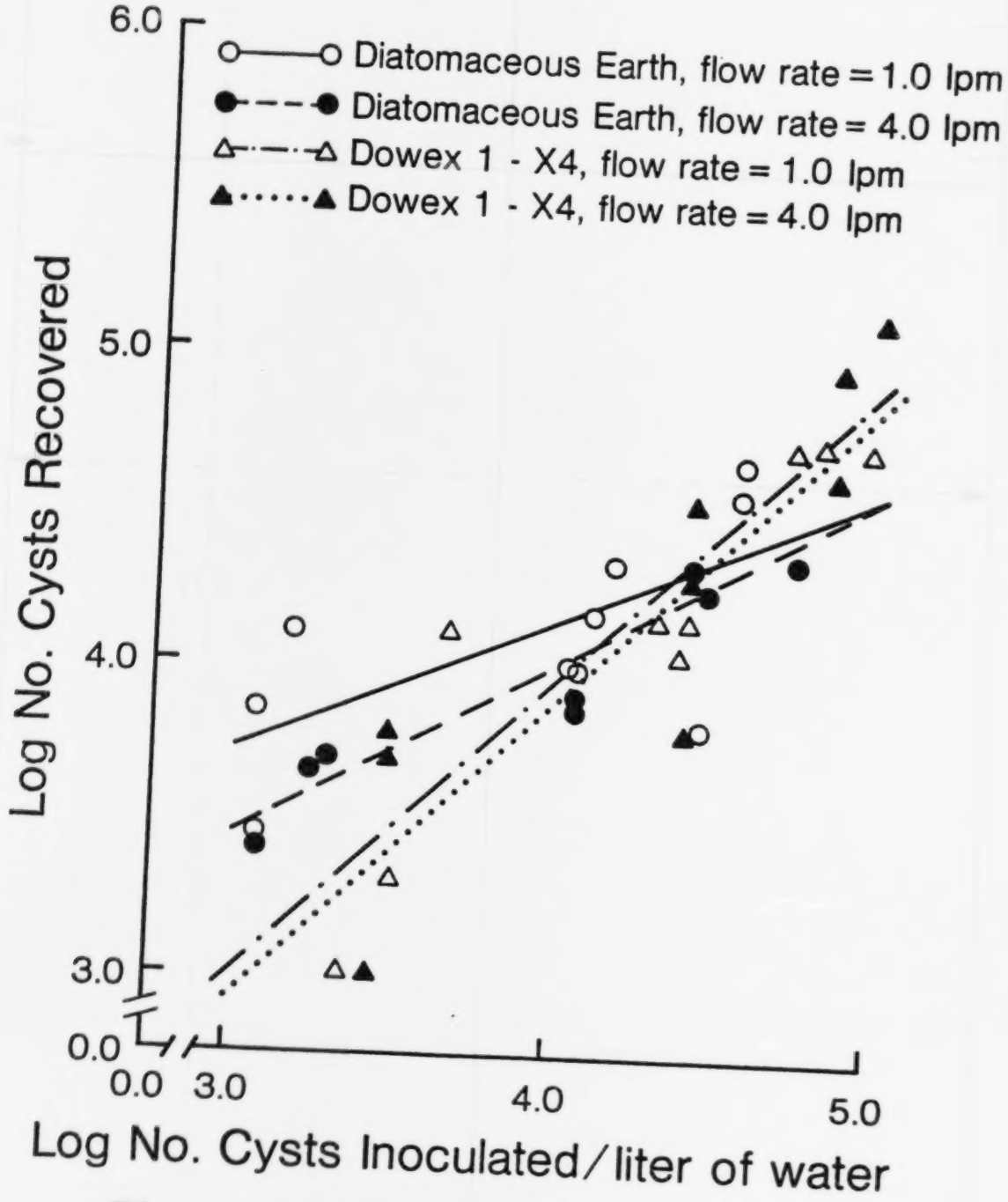


Figure 15. Cyst Recovered from Forty Liter Water Samples

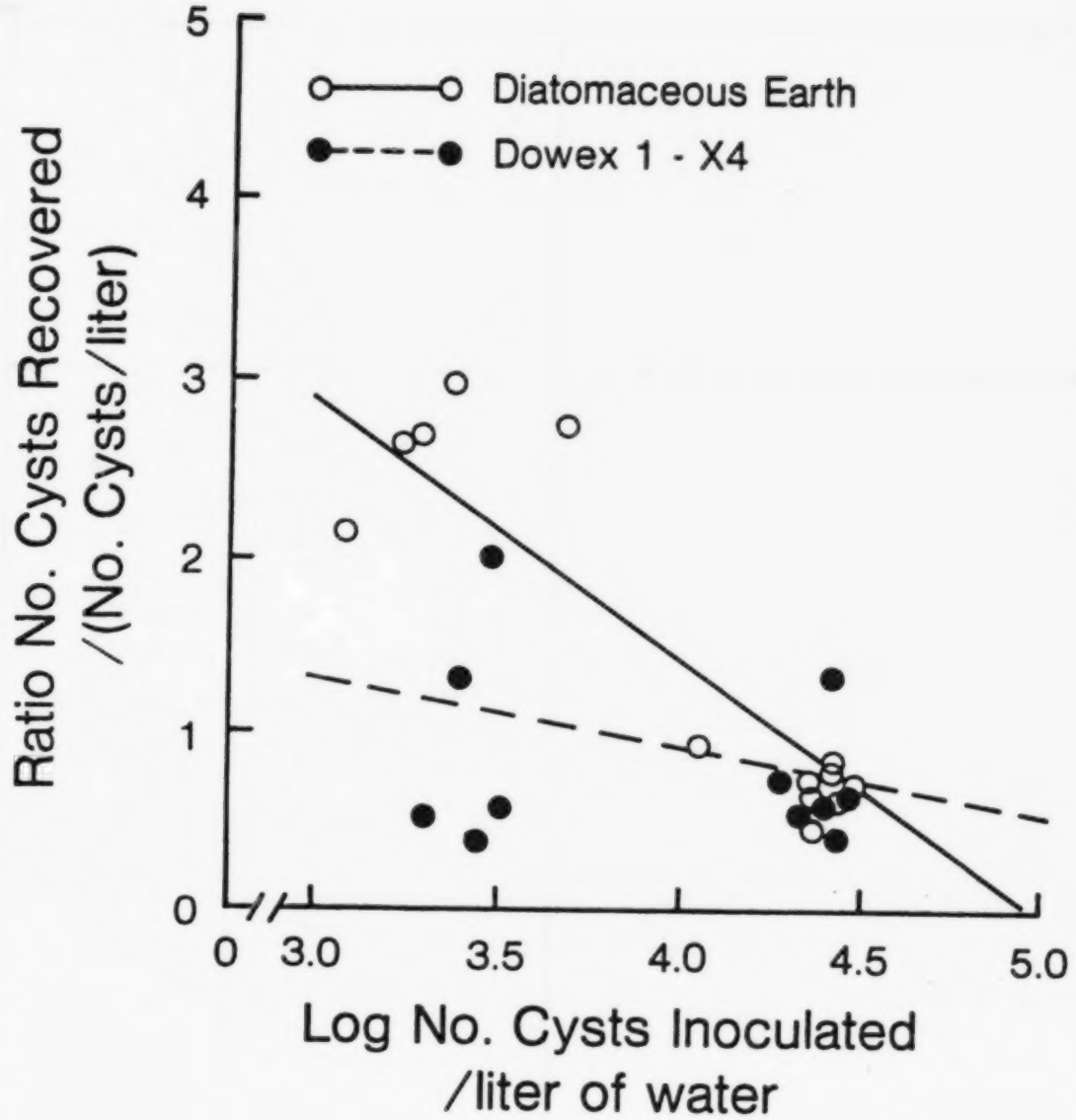


Figure 16. Ratio of Cysts Recovered to Cysts Inoculated

concentrations more likely to be encountered in water supplies, the recovery ratios of the diatomaceous earth filter were significantly higher than those observed when using the Dowex 1 - X4 sampler.

As a result of these findings, a final experiment was carried out to estimate a minimum detection limit using diatomaceous earth filters. Six 40 liter distilled water samples were inoculated with cysts to yield concentrations ranging from 25-9000 cysts/liter and filtered at a flow rate of 1 liter/min. Following filtration, each column was backwashed with 2.0 liters of distilled water at 2.0 lpm and the backwash was concentrated as previously described so that the concentrates could be examined for the presence or absence of cysts. At the inoculum concentrations used, the data was qualitative and not quantitative in that each sample was examined for the presence of cysts in the concentrate. All six samples were positive in that at least one cyst was recovered. These data suggest that if a 100 gallon water sample containing as few as 1000 cysts were sampled with the diatomaceous earth filter system, there is a good probability of detecting Giardia cysts in that water supply. The minimum detectable concentration would be approximately 10 cysts/gallon of water sampled; within the cyst density range suggested for streams contaminated by animal carriers (9).

EFFECTS OF WATERS PARAMETERS ON SAMPLING EFFICIENCY

The results of previous experiments led to the selection of diatomaceous filters as those most likely to recover cysts from water supplies. The effects of turbidity and/or pH on the ability of the sampler to capture and subsequently release cysts were determined in 12 experiments. The results are given in Table 12. Cyst recovery was significantly reduced in turbid samples when compared to non-turbid samples. A reduction of 85% in recovery was observed when water samples contained large amounts of suspended material (turbidity = 25 FTU). This reduction was accounted for in great part in that a significant portion of the suspended material was collected in the backwash concentrate making cyst detection difficult. The outcome of the experiments was dependent upon the turbidity of the water sample in that analysis of variance (ANOVA) (29) indicated that the number of cysts recovered was dependent upon the turbidity of the sample and independent of pH or inoculum concentration.

TABLE 12. Effect of Turbidity and pH on Cyst Recovery from Diatomaceous Earth Filters

Sample	Turbidity (FTU)	pH	Cyst Recovery (% of Turbid-free Sample)
1	0	5	100
2	2	6	59
3	3	7	50
4	4	9	48
5	6	5	44
6	8	6	40
7	10	7	39
8	12	9	38
9	15	5	28
10	20	6	22
11	25	7	15
12	25	9	14

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